

jc630 U.S. PTO  
02/16/99

Atty Dkt No. 6131.US.02  
0200-0030.01  
PATENT

jc511 U.S. PTO  
09/250883  
02/16/99

## REQUEST FOR FILING CONTINUATION/DIVISIONAL APPLICATION

Box Patent Application  
Assistant Commissioner for Patents  
Washington, D.C. 20231

☒ "Express Mail" Mailing Label No. EL273158376<sup>45</sup> Date of Deposit 2/16/99  
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PATRICIA K HIMERES  
(Typed or Printed Name of Person Mailing Paper or Fee)

Patricia K Himeres  
(Signature of Person Mailing Paper or Fee)

Sir:

This is a request for filing a X continuation \_\_\_ divisional application under 37 C.F.R.  
1.53(b)(1), of pending prior application serial no. 08/889,316 filed on July 8, 1997, of John C.  
RUSSELL and Tracey L. COLPITTS for REAGENTS AND METHODS USEFUL FOR  
DETECTING DISEASES OF THE BREAST.

1. X Enclosed is an application filed under 37 C.F.R. 1.53(b)(1). No new matter has been added.
2. \_\_\_ \_\_\_ verified statement(s) of small entity status  
\_\_\_ enclosed.  
\_\_\_ filed in prior application serial no. \_\_\_ filed on .
3. X The filing fee is calculated below (based on the accompanying Preliminary Amendment):

A. Basic Application Fee		\$760.00
B. Total Claims 16- 20 = 0	x \$18.00	0
C. Independent Claims 4- 3 = 1	x \$78.00	78.00
D. If multiple claims present, add	\$260.00	0
E. Total Application Fee (Total of A, B, C & D)	=	<u>838.00</u>
F. If verified statement of small entity status is enclosed, reduce Total Application Fee by 50%		0.00

G. Application Fee Due (E - F)	=	<u>838.00</u>
H. Assignment Recording Fee of \$40.00 if assignment document is enclosed	\$40.00	<u>NA</u>
I. TOTAL FEE (G + H)		838.00

4. X The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 18-1648.
5. X A check to cover the \$ 838 application fee is attached.
6.    Cancel in this application original claims of the prior application before calculating the filing fee.
7. X The inventors of the invention being claimed in this application are: Maurice Cohen, Paula N. Friedman, Michael R. Klass, Lisa Roberts-Rapp and John C. Russell.
8. X This application is being filed by less than all the inventors named in the prior application. In accordance with 37 C.F.R. 1.53(b)(1), the Commissioner is requested to delete the names of the following person who is not an inventor of the invention being claimed in this application: Tracey Colpitts.
9. X Amend the specification by inserting before the first line the sentence:  
This application is a continuation of U.S. Patent Application Serial No. 08/889,316, filed July 8, 1997.
10.    Transfer the drawings from the pending prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by a person authorized by § 1.138 and before payment of issue fee.)
- a.    New formal drawings are enclosed.
- b.    Priority of application serial no. filed on in is claimed under 35 U.S.C. 119.
- The certified copy of the priority application  
   is enclosed.  
   has been filed in prior application serial no. filed on .  
   has not yet been filed.
11. X The prior application is assigned of record to Abbott Laboratories
12. X A preliminary amendment is enclosed.

13. X Also enclosed: Declaration and Power of Attorney; Request to Use Computer Readable Form from Another Application.

14. X The power of attorney in the prior application is to

Mona Anand, Reg. No. 34,537  
Mark C. Bach, Reg. No. 34,766  
Cheryl L. Becker, Reg. No. 35,441  
Thomas D. Brainard, Reg. No. 32,459  
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David L. Weinstein, Reg. No. 28,128  
Brian L. Woodworth, Reg. No. 33,137  
Paul D. Yasger, Reg. No. 37,477  
Regina M. Anderson, Reg. No. 35,820

- a. X The power appears in the original papers in the prior application.
- b.    Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
- c.    Recognize the following individuals as Associate Attorneys/Agents :
- d. X Address all future communications to Cheryl L. Becker, Esq. at:

X Abbott Laboratories  
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377 AP6D-2  
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2/16/99  
(date)

Roberta L. Robins  
Roberta L. Robins  
Reg. No. 33,208

Address of signator:

- inventor(s)  
   assignee of complete interest  
   attorney or agent of record

X filed under § 1.34(a)

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Patricia K. Himenes  
Signature of Person Mailing Paper or Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

John C. RUSSELL et al.

Serial No.: 1.53(b) of 08/889,316      Group Art Unit: Unassigned

Filing Date: on even date herewith      Examiner: Unassigned

Title:      REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE  
BREAST

**REQUEST TO USE COMPUTER READABLE  
FORM FROM ANOTHER APPLICATION**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

The computer readable form in this application, 1.53(b) of 08/889,316 is identical with that filed in application number 08/889,316, filed July 8, 1997. In accordance with 37 CFR §1.821(e), please use the last-filed computer readable form filed in that application as the computer readable form for the instant application. It is understood that the Patent and

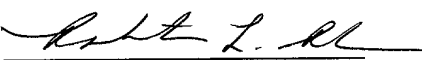
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Atty Dkt 6131.US.02  
USSN: 1.53(b) of 08/889,316  
PATENT

Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application. A paper copy of the Sequence Listing is included in the specification.

Respectfully submitted,

Date: 2/16/99

By:   
Roberta L. Robins  
Registration No. 33,208  
Attorney for Applicants

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PATRICIA K. HIMEANES

Typed or Printed Name of Person Mailing Paper or Fee

Patricia K. Himeanes

Signature of Person Mailing Paper or Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

COHEN et al.

Serial No.: 1.53(b) of 08/889,316

Art Unit: Unassigned

Filing Date: on even date herewith

Examiner: Unassigned

Title: REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF  
THE BREAST

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination the of the above-identified application, please enter the following amendments and remarks.

Amendment

In the Claims:

Please cancel claims 1-8 and add the following new claims.

--9. A purified polynucleotide derived from a BS203 nucleic acid molecule, wherein said polynucleotide has at least 50% identity with a sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof.

10. The polynucleotide of claim 9, wherein said polynucleotide specifically hybridizes to a BS203 nucleic acid sequence.

11. The polynucleotide of claim 9, wherein said polynucleotide has an overall length of about 20 to about 50 nucleotides.

12. The polynucleotide of claim 9, wherein said polynucleotide has an overall length of about 15 to about 20 nucleotides.

13. The polynucleotide of claim 9, wherein said polynucleotide is produced by recombinant techniques.

14. The polynucleotide of claim 9, wherein said polynucleotide is produced by synthetic techniques.

15. The polynucleotide of claim 9, wherein said polynucleotide comprises a sequence encoding at least one BS203 epitope.

16. The polynucleotide of claim 9, wherein said polynucleotide is attached to a solid phase.

17. The polynucleotide of claim 9, wherein said polynucleotide codes for a BS203 protein which comprises an amino acid sequence having at least 50% identity to SEQUENCE ID NO 17.

18. The polynucleotide of claim 9, wherein said polynucleotide comprises DNA having at least 50% identity with SEQUENCE ID NO 14.

19. A recombinant expression system comprising a nucleic acid sequence that includes an open reading frame derived from a BS203 polynucleotide, wherein said open reading frame is operably linked to a control sequence compatible with a desired host, and said nucleic acid sequence has at least 50% identity with a sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof.

20. A cell transfected with the recombinant expression system of claim 19.

21. A BS203 polypeptide having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof.

22. The polypeptide of claim 21, wherein said polypeptide is produced by recombinant techniques.

23. The polypeptide of claim 21, wherein said polypeptide is produced by synthetic techniques.

24. A method for producing a polypeptide comprising at least one BS203 epitope, said method comprising incubating host cells that have been transfected with an expression vector containing a polynucleotide sequence encoding a polypeptide, wherein said polypeptide comprises an amino acid sequence having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof.--

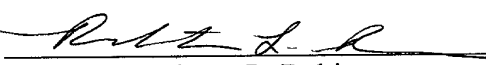


Remarks

The foregoing claims relate to polynucleotides and polypeptides derived from BS203. Support for the new claims may be found throughout the application at, e.g., page 6, lines 6-11; page 7, lines 11-17; page 8, lines 20-28; page 9, lines 13-16; page 11, line 20; and page 13, line 18. No new matter has been added by way of the foregoing new claims.

Entry of the foregoing amendments is respectfully requested.

Respectfully submitted,

Date: 2/16/99 By:   
Roberta L. Robins  
Registration No. 33,208  
Attorney for Applicants

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Application for U.S. Letters Patent Entitled

REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE BREAST

a Rule 1.53(b) Continuation of application serial no. 08/889,316 filed July 8, 1997

by Inventors:

Maurice Cohen,  
Paula N. Friedman  
Michael R. Klass  
Lisa Roberts-Rapp  
John C. Russell

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Comptroller of Patents, Washington, D.C. 20501  
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Attorney Docket No. 6131.US.02

5

REAGENTS AND METHODS USEFUL FOR  
DETECTING DISEASES OF THE BREAST

10 Background of the Invention

The invention relates generally to detecting diseases of the breast, and more particularly, relates to reagents such as polynucleotide sequences and the polypeptide sequences encoded thereby, as well as methods which utilize these sequences, which are useful for detecting, diagnosing, staging, monitoring,  
15 prognosticating, preventing or treating, or determining predisposition to diseases or conditions of the breast such as breast cancer.

Breast cancer is the most common form of cancer occurring in females in the US. The incidence of breast cancers in the United States is projected to be 180,200 cases diagnosed and 43,900 breast cancer related deaths to occur during  
20 1997 (American Cancer Society statistics). Worldwide, the incidence of breast cancer has increased from 700,000 in 1985 to about 900,000 in 1990. G.N. Hortobagyi et al., CA Cancer J Clin 45: 199-226 (1995).

Procedures used for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining predisposition to diseases  
25 or conditions of the breast such as breast cancer are of critical importance to the outcome of the patient. For example, patients diagnosed with early breast cancer have greater than a 90% five-year relative survival rate as compared to a survival rate of about 20% for patients diagnosed with distantly metastasized breast cancers. (American Cancer Society statistics). Currently, the best initial  
30 indicators of early breast cancer are physical examination of the breast and mammography. J.R. Harris et al. In: Cancer: Principles and Practice of Oncology, Fourth Edition, pp. 1264-1332, Philadelphia, PA: J/B. Lippincott Co. (1993). Mammography may detect a breast tumor before it can be detected by physical examination, but it has limitations. For example, mammography's  
35 predictive value depends on the observer's skill and the quality of the mammogram. In addition, 80 to 93% of suspicious mammograms are false

positives, and 10 to 15% of women with breast cancer have false negative mammograms. C.J. Wright *et al.*, Lancet 346: 29-32 (1995). New diagnostic methods which are more sensitive and specific for detecting early breast cancer are clearly needed.

5 Breast cancer patients are closely monitored following initial therapy and during adjuvant therapy to determine response to therapy, and to detect persistent or recurrent disease, or early distant metastasis. Current diagnostic procedures for monitoring breast cancer include mammography, bone scan, chest radiographs, liver function tests and tests for serum markers. The serum tumor  
10 markers most commonly used for monitoring patients are carcinoembryonic antigen (CEA) and CA 15-3. Limitations of CEA include absence of elevated serum levels in about 40% of women with metastatic disease. In addition, CEA elevation during adjuvant therapy may not be related to recurrence but to other factors that are not clinically important. CA 15-3 can also be negative in a  
15 significant number of patients with progressive disease and, therefore, fails to predict metastasis. Both CEA and CA 15-3 can be elevated in nonmalignant, benign conditions giving rise to false positive results. Therefore, it would be clinically beneficial to find a breast associated marker which is more sensitive and specific in detecting cancer recurrence. J. R. Harris, *et al.*, *supra*. M. K.  
20 Schwartz, In: Cancer: Principles and Practice of Oncology, Vol. 1, Fourth Edition, pp. 531 - 542, Philadelphia, PA: J/B. Lippincott Co. 1993.

Another important step in managing breast cancer is to determine the stage of the patient's disease, because it has potential prognostic value and provides criteria for designing optimal therapy. Currently, pathological staging  
25 of breast cancer is preferable over clinical staging because the former gives a more accurate prognosis. J. R. Harris, *et al.*, *supra*. On the other hand, clinical staging would be preferred were it at least as accurate as pathological staging, because it does not depend on an invasive procedure to obtain tissue for pathological evaluation. Staging of breast cancer could be improved by detecting  
30 new markers in serum or urine which could differentiate between different stages of invasion. Such markers could be mRNA or protein markers expressed by cells originating from the primary tumor in the breast but residing in blood, bone marrow or lymph nodes and could serve as sensitive indicators for metastasis to these distal organs. For example, specific protein antigens and  
35 mRNA, associated with breast epithelial cells, have been detected by immunohistochemical techniques and RT-PCR, respectively, in bone marrow,

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lymph nodes and blood of breast cancer patients suggesting metastasis. K. Pantel, *et al.*, Onkologie 18: 394-401 (1995).

Such procedures also could include assays based upon the appearance of various disease markers in test samples such as blood, plasma, serum, or urine obtained by minimally invasive procedures which are detectable by immunological methods. These procedures would provide information to aid the physician in managing the patient with disease of the breast at low cost to the patient. Markers such as prostate specific antigen (PSA) and human chorionic gonadotropin (hCG) exist and are used clinically for screening patients for prostate cancer and testicular cancer, respectively. For example, PSA normally is secreted by the prostate at high levels into the seminal fluid, but is present in very low levels in the blood of men with normal prostates. Elevated levels of PSA protein in serum are used in the early detection of prostate cancer or disease in asymptomatic men. See, for example, G.E. Hanks, *et al.*, In: *Cancer: Principles and Practice of Oncology*, Vol. 1, Fourth Edition, pp. 1073-1113, Philadelphia, PA: J.B. Lippincott Co. 1993. M. K. Schwartz, *et al.*, In: *Cancer: Principles and Practice of Oncology*, Vol. 1, Fourth Edition, pp. 531-542, Philadelphia, PA: J.B. Lippincott Co. 1993. Likewise, the management of breast diseases could be improved by the use of new markers normally expressed in the breast but found in elevated amounts in an inappropriate body compartment as a result of the disease of the breast.

Further, new markers which could predict the biologic behavior of early breast cancers would also be of significant value. Early breast cancers that threaten or will threaten the life of the patient are more clinically important than those that do not or will not be a threat. G.E. Hanks, *supra*. Such markers are needed to predict which patients with histologically negative lymph nodes will experience recurrence of cancer and also to predict which cases of ductal carcinoma in situ will develop into invasive breast carcinoma. More accurate prognostic markers would allow the clinician to accurately identify early cancers localized to the breast which will progress and metastasize if not treated aggressively. Additionally, the absence of a marker for an aggressive cancer in the patient could spare the patient expensive and non-beneficial treatment. J. R. Harris, *et al.*, *supra*. E. R. Frykberg, *et al.*, *Cancer* 74: 350-361 (1994).

It would be advantageous, therefore, to provide specific methods and reagents useful for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining predisposition to diseases or conditions of the breast. Such methods would include assaying a test sample for products of a

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gene which are overexpressed in diseases and conditions associated with the breast including cancer. Such methods may also include assaying a test sample for products of a gene which have been altered by the disease or condition associated with the breast including cancer. Such methods may further include  
5 assaying a test sample for products of a gene whose distribution among the various tissues and compartments of the body have been altered by a breast-associated disease or condition including cancer. Such methods would comprise making cDNA from mRNA in the test sample, amplifying, when necessary, portions of the cDNA corresponding to the gene or a fragment thereof, and  
10 detecting the cDNA product as an indication of the presence of the disease or condition including cancer or detecting translation products of the mRNAs comprising gene sequences as an indication of the presence of the disease. Useful reagents include polynucleotides, or fragments thereof which may be used in diagnostic methods such as reverse transcriptase-polymerase chain  
15 reaction (RT-PCR), PCR, or hybridization assays of mRNA extracted from biopsied tissue, blood or other test samples; or proteins which are the translation products of such mRNAs; or antibodies directed against these proteins. Such assays would include methods for assaying a sample for products of the gene and detecting the products as an indication of disease of the breast. Drug  
20 treatment or gene therapy for diseases and conditions of the breast including cancer can be based on these identified gene sequences or their expressed proteins, and efficacy of any particular therapy can be monitored. Furthermore, it would be advantageous to have available alternative, non-surgical diagnostic methods capable of detecting early stage breast disease such as cancer.

25

#### Summary of the Invention

The present invention provides a method of detecting a target BS203 polynucleotide in a test sample which comprises contacting the test sample with at least one BS203-specific polynucleotide and detecting the presence of the target  
30 BS203 polynucleotide in the test sample. The BS203-specific polynucleotide has at least 50% identity with a polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof. Also, the BS203-specific polynucleotide may be attached to a solid phase prior to a performing the method.

35

The present invention also provides a method for detecting BS203 mRNA in a test sample, which comprises performing reverse transcription (RT) with at least

one primer in order to produce cDNA, amplifying the cDNA so obtained using BS203 oligonucleotides as sense and antisense primers to obtain BS203 amplicon, and detecting the presence of the BS203 amplicon as an indication of the presence of BS203 mRNA in the test sample, wherein the BS203 oligonucleotides have at least  
5 50% identity to a sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof. Amplification can be performed by the polymerase chain reaction. Also, the test sample can be reacted with a solid phase prior to performing the method, prior to amplification or prior to detection. This reaction can be a direct or an indirect reaction. Further, the detection step can  
10 comprise utilizing a detectable label capable of generating a measurable signal. The detectable label can be attached to a solid phase.

The present invention further provides a method of detecting a target BS203 polynucleotide in a test sample suspected of containing target BS203 polynucleotides, which comprises (a) contacting the test sample with at least one  
15 BS203 oligonucleotide as a sense primer and at least one BS203 oligonucleotide as an anti-sense primer, and amplifying same to obtain a first stage reaction product; (b) contacting the first stage reaction product with at least one other BS203 oligonucleotide to obtain a second stage reaction product, with the proviso that the other BS203 oligonucleotide is located 3' to the BS203 oligonucleotides utilized in  
20 step (a) and is complementary to the first stage reaction product; and (c) detecting the second stage reaction product as an indication of the presence of a target BS203 polynucleotide in the test sample. The BS203 oligonucleotides selected as reagents in the method have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof.  
25 Amplification may be performed by the polymerase chain reaction. The test sample can be reacted either directly or indirectly with a solid phase prior to performing the method, or prior to amplification, or prior to detection. The detection step also comprises utilizing a detectable label capable of generating a measurable signal; further, the detectable label can be attached to a solid phase. Test kits useful for  
30 detecting target BS203 polynucleotides in a test sample further are provided which comprise a container containing at least one BS203 specific polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof. These test kits further comprise containers with tools useful for collecting test samples (such as blood, urine, saliva and stool). Such tools  
35 include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; and cups for collecting and stabilizing

urine or stool samples. Collection materials such as, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens.

The present invention provides a polypeptide encoded by BS203. The polypeptide can be produced by recombinant technology, provided in purified form, or produced by synthetic techniques. The polypeptide comprises an amino acid sequence which has at least 50% identity to an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, and SEQUENCE ID NO 21.

Also provided is an antibody which specifically binds to at least one BS203 epitope. The antibody can be a polyclonal or monoclonal antibody. The epitope is derived from an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof. Assay kits for determining the presence of BS203 antigen or anti-BS203 antibody in a test sample are also included. In one embodiment, the assay kits comprise a container containing at least one BS203 polypeptide having at least 50% identity to an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof. Further, the test kit can comprise a container with tools useful for collecting test samples (such as blood, urine, saliva and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; and cups for collecting and stabilizing urine or stool samples. Collection materials such as, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. These collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. Also, the polypeptide can be attached to a solid phase.

Another assay kit for determining the presence of BS203 antigen or anti-BS203 antibody in a test sample comprises a container containing an antibody which specifically binds to a BS203 antigen, wherein the BS203 antigen comprises at least one BS203-encoded epitope. The BS203 antigen has at least about 60% sequence similarity to a sequence of a BS203-encoded antigen selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO



19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof. These test kits can further comprise containers with tools useful for collecting test samples (such as blood, urine, saliva and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. These collection materials also may be treated with, or contain, preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. The antibody can be attached to a solid phase.

A method for producing a polypeptide which contains at least one epitope of BS203 is provided, which method comprises incubating host cells transfected with an expression vector. This vector comprises a polynucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence having at least 50% identity to a BS203 amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof.

A method for detecting BS203 antigen in a test sample suspected of containing BS203 antigen also is provided. The method comprises contacting the test sample with an antibody or fragment thereof which specifically binds to at least one epitope of a BS203 antigen, for a time and under conditions sufficient for the formation of antibody/antigen complexes; and detecting the presence of such complexes containing the antibody as an indication of the presence of BS203 antigen in the test sample. The antibody can be attached to a solid phase and be either a monoclonal or polyclonal antibody. Furthermore, the antibody specifically binds to at least one BS203 antigen selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof.

Another method is provided which detects antibodies which specifically bind to BS203 antigen in a test sample suspected of containing these antibodies. The method comprises contacting the test sample with a polypeptide which contains at least one BS203 epitope, wherein the BS203 epitope comprises an amino acid sequence having at least 50% identity with an amino acid sequence encoded by a BS203 polynucleotide, or a fragment thereof. Contacting is carried out for a time and under conditions sufficient to allow antigen/antibody complexes to form. The method further entails detecting complexes which contain the polypeptide. The

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polypeptide can be attached to a solid phase. Further, the polypeptide can be a recombinant protein or a synthetic peptide having at least 50% identity to an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof.

A method for producing antibodies to BS203 antigen also is provided, which method comprises administering to an individual an isolated immunogenic polypeptide or fragment thereof, wherein the isolated immunogenic polypeptide comprises at least one BS203 epitope in an amount sufficient to produce an immune response. The isolated, immunogenic polypeptide comprises an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof.

Another method for producing antibodies which specifically bind to BS203 antigen is disclosed, which comprises administering to a mammal a plasmid comprising a nucleic acid sequence which encodes at least one BS203 epitope derived from an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof.

Also provided is a composition of matter that comprises a polypeptide with at least one BS203 epitope of about 8-10 amino acids. The polypeptide comprises an amino acid sequence having at least 90% identity to an amino acid sequence selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof. Also provided is a gene or fragment thereof coding for a BS203 polypeptide which has at least 50% identity to SEQUENCE ID NO 17, and a gene or a fragment thereof comprising DNA having at least 50% identity to SEQUENCE ID NO 14.

### Brief Description of the Drawings

FIGURES 1A-1C show the nucleotide alignment of clones 2269559 (SEQUENCE ID NO 1), 1664718 (SEQUENCE ID NO 2), 2360586 (SEQUENCE ID NO 3), 1436565 (SEQUENCE ID NO 4), 2479125 (SEQUENCE ID NO 5), 2247228 (SEQUENCE ID NO 6), 2112334 (SEQUENCE ID NO 7), 960106 (SEQUENCE ID NO 8), 962045 (SEQUENCE ID NO 9), 959580 (SEQUENCE ID NO 10), 961381 (SEQUENCE ID NO 11), 2517547 (SEQUENCE ID NO 12),

2124915 (SEQUENCE ID NO 13) and the consensus sequence (SEQUENCE ID NO 14) derived therefrom.

FIGURE 2 shows the contig map depicting the formation of the consensus nucleotide sequence (SEQUENCE ID NO 14) from the nucleotide alignment of overlapping clones 2269559 (SEQUENCE ID NO 1), 1664718 (SEQUENCE ID NO 2), 2360586 (SEQUENCE ID NO 3), 1436565 (SEQUENCE ID NO 4), 2479125 (SEQUENCE ID NO 5), 2247228 (SEQUENCE ID NO 6), 2112334 (SEQUENCE ID NO 7), 960106 (SEQUENCE ID NO 8), 962045 (SEQUENCE ID NO 9), 959580 (SEQUENCE ID NO 10), 961381 (SEQUENCE ID NO 11), 2517547 (SEQUENCE ID NO 12), and 2124915 (SEQUENCE ID NO 13).

#### Detailed Description of the Invention

The present invention provides a gene or a fragment thereof which codes for a BS203 polypeptide having at least about 50% identity to SEQUENCE ID NO 17. The present invention further encompasses a BS203 gene or a fragment thereof comprising DNA which has at least about 50% identity to SEQUENCE ID NO 14.

The present invention provides methods for assaying a test sample for products of a breast tissue gene designated as BS203, which comprises making cDNA from mRNA in the test sample, and detecting the cDNA as an indication of the presence of breast tissue gene BS203. The method may include an amplification step, wherein one or more portions of the mRNA from BS203 corresponding to the gene or fragments thereof is amplified. Methods also are provided for assaying for the translation products of BS203. Test samples which may be assayed by the methods provided herein include tissues, cells, body fluids and secretions. The present invention also provides reagents such as oligonucleotide primers and polypeptides which are useful in performing these methods.

Portions of the nucleic acid sequences disclosed herein are useful as primers for the reverse transcription of RNA or for the amplification of cDNA; or as probes to determine the presence of certain mRNA sequences in test samples. Also disclosed are nucleic acid sequences which permit the production of encoded polypeptide sequences which are useful as standards or reagents in diagnostic immunoassays, as targets for pharmaceutical screening assays and/or as components or as target sites for various therapies. Monoclonal and polyclonal antibodies directed against at least one epitope contained within these polypeptide sequences are useful as delivery agents for therapeutic agents as well as for diagnostic tests and for screening for diseases or conditions associated with BS203, especially breast cancer.

Isolation of sequences of other portions of the gene of interest can be accomplished utilizing probes or PCR primers derived from these nucleic acid sequences. This allows additional probes of the mRNA or cDNA of interest to be established, as well as corresponding encoded polypeptide sequences. These additional molecules are  
5 useful in detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to, diseases and conditions of the breast such as breast cancer, characterized by BS203 as disclosed herein.

Techniques for determining amino acid sequence "similarity" are well-known in the art. In general, "similarity" means the exact amino acid to amino acid  
10 comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining  
15 the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more  
20 polynucleotide sequences can be compared by determining their "percent identity." Two or more amino acid sequences likewise can be compared by determining their "percent identity." The programs available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics Computer Group, Madison, WI), for example, the GAP program, are capable of calculating both the identity between two  
25 polynucleotides and the identity and similarity between two polypeptide sequences, respectively. Other programs for calculating identity or similarity between sequences are known in the art.

The compositions and methods described herein will enable the identification of certain markers as indicative of a breast tissue disease or condition; the  
30 information obtained therefrom will aid in the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining diseases or conditions associated with BS203, especially breast cancer. Test methods include, for example, probe assays which utilize the sequence(s) provided herein and which also may utilize nucleic acid amplification methods such as the polymerase chain  
35 reaction (PCR), the ligase chain reaction (LCR); and hybridization. In addition, the nucleotide sequences provided herein contain open reading frames from which an

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immunogenic epitope may be found. This epitope is believed to be unique to the disease state or condition associated with BS203. It also is thought that the polynucleotides or polypeptides and protein encoded by the BS203 gene are useful as a marker. This marker is either elevated in disease such as breast cancer, altered in disease such as breast cancer, or present as a normal protein but appearing in an inappropriate body compartment. The uniqueness of the epitope may be determined by (i) its immunological reactivity and specificity with antibodies directed against proteins and polypeptides encoded by the BS203 gene, and (ii) its nonreactivity with any other tissue markers. Methods for determining immunological reactivity are well-known and include but are not limited to, for example, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), hemagglutination (HA), fluorescence polarization immunoassay (FPIA); chemiluminescent immunoassay (CLIA) and others; several examples of suitable methods are described herein.

Unless otherwise stated, the following terms shall have the following meanings:

A polynucleotide "derived from" or "specific for" a designated sequence refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region of the designated nucleotide sequence. The sequence may be complementary or identical to a sequence which is unique to a particular polynucleotide sequence as determined by techniques known in the art. Comparisons to sequences in databanks, for example, can be used as a method to determine the uniqueness of a designated sequence. Regions from which sequences may be derived, include but are not limited to, regions encoding specific epitopes, as well as non-translated and/or non-transcribed regions.

The derived polynucleotide will not necessarily be derived physically from the nucleotide sequence of interest under study, but may be generated in any manner, including but not limited to chemical synthesis, replication, reverse transcription or transcription, which is based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived. As such, it may represent either a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with the intended use.

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5 A "fragment" of a specified polynucleotide refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region of the specified nucleotide sequence.

10 The term "primer" denotes a specific oligonucleotide sequence which is complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase, RNA polymerase or reverse transcriptase.

15 The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, e.g., PNA as defined hereinbelow) which can be used to identify a specific polynucleotide present in samples bearing the complementary sequence.

20 "Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide encoded by the sequence. Thus, a "polypeptide," "protein," or "amino acid" sequence has at least about 50% identity, preferably about 60% identity, more preferably about 75-85% identity, and most preferably about 90-95% or more identity to a BS203 amino acid sequence. Further, the BS203 "polypeptide," "protein," or "amino acid" sequence may have at least about 60% similarity, preferably at least about 75% similarity, more preferably about 85% similarity, and most preferably about 95% or more similarity to a polypeptide or amino acid sequence of BS203. This amino acid sequence can be selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof.

30 A "recombinant polypeptide," "recombinant protein," or "a polypeptide produced by recombinant techniques," which terms may be used interchangeably herein, describes a polypeptide which by virtue of its origin or manipulation is not associated with all or a portion of the polypeptide with which it is associated in nature and/or is linked to a polypeptide other than that to which it is linked in nature. A recombinant or encoded polypeptide or protein is not necessarily translated from a

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designated nucleic acid sequence. It also may be generated in any manner, including chemical synthesis or expression of a recombinant expression system.

The term "synthetic peptide" as used herein means a polymeric form of amino acids of any length, which may be chemically synthesized by methods well-known to the routineer. These synthetic peptides are useful in various applications.

The term "polynucleotide" as used herein means a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes double- and single-stranded DNA, as well as, double- and single-stranded RNA. It also includes modifications, such as methylation or capping and unmodified forms of the polynucleotide. The terms "polynucleotide," "oligomer," "oligonucleotide," and "oligo" are used interchangeably herein.

"A sequence corresponding to a cDNA" means that the sequence contains a polynucleotide sequence that is identical or complementary to a sequence in the designated DNA. The degree (or "percent") of identity or complementarity to the cDNA will be approximately 50% or greater, preferably at least about 70% or greater, and more preferably at least about 90% or greater. The sequence that corresponds to the identified cDNA will be at least about 50 nucleotides in length, preferably at least about 60 nucleotides in length, and more preferably at least about 70 nucleotides in length. The correspondence between the gene or gene fragment of interest and the cDNA can be determined by methods known in the art and include, for example, a direct comparison of the sequenced material with the cDNAs described, or hybridization and digestion with single strand nucleases, followed by size determination of the digested fragments.

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

"Purified polypeptide" or "purified protein" means a polypeptide of interest or fragment thereof which is essentially free of, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, cellular

components with which the polypeptide of interest is naturally associated. Methods for purifying polypeptides of interest are known in the art.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, which is separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

"Polypeptide" and "protein" are used interchangeably herein and indicate at least one molecular chain of amino acids linked through covalent and/or non-covalent bonds. The terms do not refer to a specific length of the product. Thus peptides, oligopeptides and proteins are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. In addition, protein fragments, analogs, mutated or variant proteins, fusion proteins and the like are included within the meaning of polypeptide.

A "fragment" of a specified polypeptide refers to an amino acid sequence which comprises at least about 3-5 amino acids, more preferably at least about 8-10 amino acids, and even more preferably at least about 15-20 amino acids derived from the specified polypeptide.

"Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transferred DNA, and include the original progeny of the original cell which has been transfected.

As used herein "replicon" means any genetic element, such as a plasmid, a chromosome or a virus, that behaves as an autonomous unit of polynucleotide replication within a cell.

A "vector" is a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment.

The term "control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include promoter, ribosomal binding



site and terminators; in eukaryotes, such control sequences generally include promoters, terminators and, in some instances, enhancers. The term "control sequence" thus is intended to include at a minimum all components whose presence is necessary for expression, and also may include additional components whose presence is advantageous, for example, leader sequences.

"Operably linked" refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for example, a control sequence "operably linked" to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "open reading frame" or "ORF" refers to a region of a polynucleotide sequence which encodes a polypeptide. This region may represent a portion of a coding sequence or a total coding sequence.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5' -terminus and a translation stop codon at the 3' -terminus. A coding sequence can include, but is not limited to, mRNA, cDNA and recombinant polynucleotide sequences.

The term "immunologically identifiable with/as" refers to the presence of epitope(s) and polypeptide(s) which also are present in and are unique to the designated polypeptide(s). Immunological identity may be determined by antibody binding and/or competition in binding. These techniques are known to the routineer and also are described herein. The uniqueness of an epitope also can be determined by computer searches of known data banks, such as GenBank, for the polynucleotide sequences which encode the epitope and by amino acid sequence comparisons with other known proteins.

As used herein, "epitope" means an antigenic determinant of a polypeptide or protein. Conceivably, an epitope can comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids and more usually, it consists of at least eight to ten amino acids. Methods of examining spatial conformation are known in the art and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

A "conformational epitope" is an epitope that is comprised of specific juxtaposition of amino acids in an immunologically recognizable structure, such

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A polypeptide is “immunologically reactive” with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly by the kinetics of antibody binding, and/or by competition in binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The methods for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

The term “transfection” refers to the introduction of an exogenous polynucleotide into a prokaryotic or eucaryotic host cell, irrespective of the method used for the introduction. The term “transfection” refers to both stable and transient introduction of the polynucleotide, and encompasses direct uptake of polynucleotides, transformation, transduction, and f-mating. Once introduced into the host cell, the exogenous polynucleotide may be maintained as a non-integrated replicon, for example, a plasmid, or alternatively, may be integrated into the host genome.

The term “individual” as used herein refers to vertebrates, particularly members of the mammalian species and includes but is not limited to domestic animals, sports animals, primates and humans; more particularly the term refers to humans.

The term “test sample” refers to a component of an individual's body which is the source of the analyte (such as, antibodies of interest or antigens of interest). These components are well known in the art. A test sample is typically anything suspected of containing a target sequence. Test samples can be prepared using methodologies well known in the art such as by obtaining a specimen from an individual and, if necessary, disrupting any cells contained thereby to release target

5 nucleic acids. These test samples include biological samples which can be tested by the methods of the present invention described herein and include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, sputum, bronchial washing, bronchial aspirates, urine, lymph fluids and various external secretions of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk, white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; tissue specimens which may be fixed; and cell specimens which may be fixed.

10 "Purified product" refers to a preparation of the product which has been isolated from the cellular constituents with which the product is normally associated and from other types of cells which may be present in the sample of interest.

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"PNA" denotes a "peptide nucleic acid analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. "MA" denotes a "morpholino analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. See, for example, U.S. Patent No. 5,378,841, which is incorporated herein by reference. PNAs are neutrally charged moieties which can be directed against RNA targets or DNA. PNA probes used in assays in place of, for example, the DNA probes of the present invention, offer advantages not achievable when DNA probes are used. These advantages include manufacturability, large scale labeling, reproducibility, stability, insensitivity to changes in ionic strength and resistance to enzymatic degradation which is present in methods utilizing DNA or RNA. These PNAs can be labeled with ("attached to") such signal generating compounds as fluorescein, radionucleotides, chemiluminescent compounds and the like. PNAs or other nucleic acid analogs such as MAs thus can be used in assay methods in place of DNA or RNA. Although assays are described herein utilizing DNA probes, it is within the scope of the routineer that PNAs or MAs can be substituted for RNA or DNA with appropriate changes if and as needed in assay reagents.

25 "Analyte," as used herein, is the substance to be detected which may be present in the test sample. The analyte can be any substance for which there exists a naturally occurring specific binding member (such as, an antibody), or for which a specific binding member can be prepared. Thus, an analyte is a substance that can bind to one or more specific binding members in an assay. "Analyte" also includes any antigenic substances, haptens, antibodies and combinations thereof. As a member of a specific binding pair, the analyte can be detected by means of naturally occurring specific binding partners (pairs) such as the use of intrinsic factor protein

as a member of a specific binding pair for the determination of Vitamin B12, the use of folate-binding protein to determine folic acid, or the use of a lectin as a member of a specific binding pair for the determination of a carbohydrate. The analyte can include a protein, a polypeptide, an amino acid, a nucleotide target and the like.

5           “Diseases of the Breast” or “Breast disease,” or “condition of the breast” as used herein, refer to any disease or condition of the breast including, but not limited to, atypical hyperplasia, fibroadenoma, cystic breast disease, and cancer.

          “Breast cancer,” as used herein, refers to any malignant disease of the breast including, but not limited to, ductal carcinoma in situ, lobular carcinoma in situ,  
10   infiltrating ductal carcinoma, medullary carcinoma, tubular carcinoma, mucinous carcinoma, infiltrating lobular carcinoma, infiltrating comedocarcinoma and inflammatory carcinoma.

          An “Expressed Sequence Tag” or “EST” refers to the partial sequence of a cDNA insert which has been made by reverse transcription of mRNA extracted from  
15   a tissue followed by insertion into a vector.

          A “transcript image” refers to a table or list giving the quantitative distribution of ESTs in a library and represents the genes active in the tissue from which the library was made.

          The present invention provides assays which utilize specific binding  
20   members. A “specific binding member,” as used herein, is a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins,  
25   complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, antibodies and antibody fragments,  
30   both monoclonal and polyclonal and complexes thereof, including those formed by recombinant DNA molecules.

          The term “hapten,” as used herein, refers to a partial antigen or non-protein binding member which is capable of binding to an antibody, but which is not capable of eliciting antibody formation unless coupled to a carrier protein.

35           A “capture reagent,” as used herein, refers to an unlabeled specific binding member which is specific either for the analyte as in a sandwich assay, for the

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Abbott Park, IL) and others. The "solid phase" is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips, sheep (or other suitable animal's) red blood cells and Duracytes® are all  
5 suitable examples. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A "solid phase," as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an  
10 additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid phase and which  
15 has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead,  
20 microparticle, chip, sheep (or other suitable animal's) red blood cells, Duracytes® and other configurations known to those of ordinary skill in the art.

It is contemplated and within the scope of the present invention that the solid phase also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens.  
25 Microporous structure generally are preferred, but materials with gel structure in the hydrated state may be used as well. Such useful solid supports include but are not limited to nitrocellulose and nylon. It is contemplated that such porous solid supports described herein preferably are in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1 mm. The pore size may vary within  
30 wide limits and preferably is from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surface of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces.  
35 Other suitable solid supports are known in the art.

Reagents.

The present invention provides reagents such as polynucleotide sequences derived from a breast tissue of interest and designated as BS203, polypeptides encoded thereby and antibodies specific for these polypeptides. The present invention also provides reagents such as oligonucleotide fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to these polynucleotides. The polynucleotides, polypeptides, or antibodies of the present invention may be used to provide information leading to the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating of, or determining the predisposition to, diseases and conditions of the breast such as cancer. The sequences disclosed herein represent unique polynucleotides which can be used in assays or for producing a specific profile of gene transcription activity. Such assays are disclosed in European Patent Number 0373203B1 and International Publication No. WO 95/11995, which are hereby incorporated by reference.

Selected BS203-derived polynucleotides can be used in the methods described herein for the detection of normal or altered gene expression. Such methods may employ BS203 polynucleotides or oligonucleotides, fragments or derivatives thereof, or nucleic acid sequences complementary thereto.

The polynucleotides disclosed herein, their complementary sequences, or fragments of either, can be used in assays to detect, amplify or quantify genes, nucleic acids, cDNAs or mRNAs relating to breast tissue disease and conditions associated therewith. They also can be used to identify an entire or partial coding region of a BS203 polypeptide. They further can be provided in individual containers in the form of a kit for assays, or provided as individual compositions. If provided in a kit for assays, other suitable reagents such as buffers, conjugates and the like may be included.

The polynucleotide may be in the form of RNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, nucleic acid analogs and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding (sense) strand or non-coding (anti-sense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA provided herein.

This polynucleotide may include only the coding sequence for the polypeptide, or the coding sequence for the polypeptide and additional coding

5 In addition, the invention includes variant polynucleotides containing modifications such as polynucleotide deletions, substitutions or additions; and any polypeptide modification resulting from the variant polynucleotide sequence. A polynucleotide of the present invention also may have a coding sequence which is a naturally occurring allelic variant of the coding sequence provided herein.

25 The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein. See, for example, I. Wilson, et al., Cell 37:767 (1984).

The present invention also provides an antibody produced by using a purified BS203 polypeptide of which at least a portion of the polypeptide is encoded by a BS203 polynucleotide selected from the polynucleotides provided herein. These antibodies may be used in the methods provided herein for the detection of BS203



antigen in test samples. The presence of BS203 antigen in the test samples is indicative of the presence of a breast disease or condition. The antibody also may be used for therapeutic purposes, for example, in neutralizing the activity of BS203 polypeptide in conditions associated with altered or abnormal expression.

5           The present invention further relates to a BS203 polypeptide which has the deduced amino acid sequence as provided herein, as well as fragments, analogs and derivatives of such polypeptide. The polypeptide of the present invention may be a recombinant polypeptide, a natural purified polypeptide or a synthetic polypeptide. The fragment, derivative or analog of the BS203 polypeptide may be one in which  
10   one or more of the amino acid residues is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or it may be one in which one or more of the amino acid residues includes a substituent group; or it may be one in which the polypeptide is fused with another  
15   compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or it may be one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are within the scope of the present  
20   invention. The polypeptides and polynucleotides of the present invention are provided preferably in an isolated form and preferably purified.

          Thus, a polypeptide of the present invention may have an amino acid sequence that is identical to that of the naturally occurring polypeptide or that is different by minor variations due to one or more amino acid substitutions. The  
25   variation may be a "conservative change" typically in the range of about 1 to 5 amino acids, wherein the substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine or threonine with serine. In contrast, variations may include nonconservative changes, e.g., replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid  
30   deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without changing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR Inc., Madison WI).

35           Probes constructed according to the polynucleotide sequences of the present invention can be used in various assay methods to provide various types of analysis.

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For example, such probes can be used in fluorescent in situ hybridization (FISH) technology to perform chromosomal analysis, and used to identify cancer-specific structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR-generated and/or allele specific oligonucleotides probes, allele specific amplification or by direct sequencing. Probes also can be labeled with radioisotopes, directly- or indirectly-detectable haptens, or fluorescent molecules, and utilized for in situ hybridization studies to evaluate the mRNA expression of the gene comprising the polynucleotide in tissue specimens or cells.

10 This invention also provides teachings as to the production of the polynucleotides and polypeptides provided herein.

#### Probe Assays

15 The sequences provided herein may be used to produce probes which can be used in assays for the detection of nucleic acids in test samples. The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The design of such probes for optimization in assays is within the skill of the routineer. Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multi-gene family or in related species like mouse and man.

25 The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In PCR, a pair of primers are employed in excess to hybridize to the complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation from the original target strand. New primers then are hybridized and extended by a polymerase, and the cycle is repeated to geometrically increase the number of target sequence molecules. PCR is disclosed in U.S. Patents 4,683,195 and 4,683,202, which are incorporated herein by reference.

35 The Ligase Chain Reaction (LCR) is an alternate method for nucleic acid amplification. In LCR, probe pairs are used which include two primary (first and second) and two secondary (third and fourth) probes, all of which are employed in molar excess to target. The first probe hybridizes to a first segment of the target

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strand and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3' hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. This technique is described more completely in EP-A- 320 308 to K. Backman published June 16, 1989 and EP-A- 439 182 to K. Backman et al, published July 31, 1991, both of which are incorporated herein by reference.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770, which is incorporated herein by reference; or reverse transcribe mRNA into cDNA followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by R.L. Marshall, et al., PCR Methods and Applications 4: 80-84 (1994), which also is incorporated herein by reference.

Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described by J.C. Guatelli, et al., PNAS USA 87:1874-1878 (1990) and also described by J. Compton, Nature 350 (No. 6313):91-92 (1991); Q-beta amplification as described in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G.T. Walker et al., Clin. Chem. 42:9-13 [1996]) and European Patent Application No. 684315; and target mediated amplification, as described in International Publication No. WO 93/22461.

Detection of BS203 may be accomplished using any suitable detection method, including those detection methods which are currently well known in the art, as well as detection strategies which may evolve later. Examples of the forgoing presently known detection methods are hereby incorporated herein by reference. See, for example, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S.

Patent No. 5,210,015. Examples of such detection methods include target amplification methods as well as signal amplification technologies. An example of presently known detection methods would include the nucleic acid amplification technologies referred to as PCR, LCR, NASBA, SDA, RCR and TMA. See, for example, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015. All of the foregoing are hereby incorporated by reference. Detection may also be accomplished using signal amplification such as that disclosed in Snitman et al., U.S. Patent No. 5,273,882. While the amplification of target or signal is preferred at present, it is contemplated and within the scope of the present invention that ultrasensitive detection methods which do not require amplification can be utilized herein.

Detection, both amplified and non-amplified may be (combined) carried out using a variety of heterogeneous and homogeneous detection formats. Examples of heterogeneous detection formats are disclosed in Snitman et al., U.S. Patent No. 5,273,882, Albarella et al in EP-84114441.9, Urdea et al., U.S. Patent No. 5,124,246, Ullman et al. U.S. Patent No. 5,185,243 and Kourilsky et al., U.S. Patent No. 4,581,333. All of the foregoing are hereby incorporated by reference. Examples of homogeneous detection formats are disclosed in, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015, which are incorporated herein by reference. It also is contemplated and within the scope of the present invention is the use of multiple probes in the hybridization assay which improves sensitivity and amplification of the BS203 signal. See, for example, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015, which are incorporated herein by reference.

In one embodiment, the present invention generally comprises the steps of contacting a test sample suspected of containing a target polynucleotide sequence with amplification reaction reagents comprising an amplification primer, and a detection probe that can hybridize with an internal region of the amplicon sequences. Probes and primers employed according to the method provided herein are labeled with capture and detection labels, wherein probes are labeled with one type of label and primers are labeled with the other type of label. Additionally, the primers and probes are selected such that the probe sequence has a lower melt temperature than the primer sequences. The amplification reagents, detection reagents and test sample are placed under amplification conditions whereby, in the presence of target sequence, copies of the target sequence (an amplicon) are produced. In the usual case, the amplicon is double stranded because primers are provided to amplify a

target sequence and its complementary strand. The double stranded amplicon then is thermally denatured to produce single stranded amplicon members. Upon formation of the single stranded amplicon members, the mixture is cooled to allow the formation of complexes between the probes and single stranded amplicon members.

5       As the single stranded amplicon sequences and probe sequences are cooled, the probe sequences preferentially bind the single stranded amplicon members. This finding is counterintuitive given that the probe sequences generally are selected to be shorter than the primer sequences and therefore have a lower melt temperature than the primers. Accordingly, the melt temperature of the amplicon produced by the  
10       primers should also have a higher melt temperature than the probes. Thus, as the mixture cools, the re-formation of the double stranded amplicon would be expected. As previously stated, however, this is not the case. The probes are found to preferentially bind the single stranded amplicon members. Moreover, this preference of probe/single stranded amplicon binding exists even when the primer sequences  
15       are added in excess of the probes.

After the probe/single stranded amplicon member hybrids are formed, they are detected. Standard heterogeneous assay formats are suitable for detecting the hybrids using the detection labels and capture labels present on the primers and probes. The hybrids can be bound to a solid phase reagent by virtue of the capture  
20       label and detected by virtue of the detection label. In cases where the detection label is directly detectable, the presence of the hybrids on the solid phase can be detected by causing the label to produce a detectable signal, if necessary, and detecting the signal. In cases where the label is not directly detectable, the captured hybrids can be contacted with a conjugate, which generally comprises a binding member attached  
25       to a directly detectable label. The conjugate becomes bound to the complexes and the conjugates presence on the complexes can be detected with the directly detectable label. Thus, the presence of the hybrids on the solid phase reagent can be determined. Those skilled in the art will recognize that wash steps may be employed to wash away unhybridized amplicon or probe as well as unbound conjugate.

30       Although the target sequence is described as single stranded, it also is contemplated to include the case where the target sequence is actually double stranded but is merely separated from its complement prior to hybridization with the amplification primer sequences. In the case where PCR is employed in this method, the ends of the target sequences are usually known. In cases where LCR or a  
35       modification thereof is employed in the preferred method, the entire target sequence

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is usually known. Typically, the target sequence is a nucleic acid sequence such as, for example, RNA or DNA.

The method provided herein can be used in well-known amplification reactions that include thermal cycle reaction mixtures, particularly in PCR and gap LCR (GLCR). Amplification reactions typically employ primers to repeatedly generate copies of a target nucleic acid sequence, which target sequence is usually a small region of a much larger nucleic acid sequence. Primers are themselves nucleic acid sequences that are complementary to regions of a target sequence. Under amplification conditions, these primers hybridize or bind to the complementary regions of the target sequence. Copies of the target sequence typically are generated by the process of primer extension and/or ligation which utilizes enzymes with polymerase or ligase activity, separately or in combination, to add nucleotides to the hybridized primers and/or ligate adjacent probe pairs. The nucleotides that are added to the primers or probes, as monomers or preformed oligomers, are also complementary to the target sequence. Once the primers or probes have been sufficiently extended and/or ligated they are separated from the target sequence, for example, by heating the reaction mixture to a "melt temperature" which is one in which complementary nucleic acid strands dissociate. Thus, a sequence complementary to the target sequence is formed.

A new amplification cycle then can take place to further amplify the number of target sequences by separating any double stranded sequences, allowing primers or probes to hybridize to their respective targets, extending and/or ligating the hybridized primers or probes and re-separating. The complementary sequences that are generated by amplification cycles can serve as templates for primer extension or filling the gap of two probes to further amplify the number of target sequences. Typically, a reaction mixture is cycled between 20 and 100 times, more typically, a reaction mixture is cycled between 25 and 50 times. The numbers of cycles can be determined by the routineer. In this manner, multiple copies of the target sequence and its complementary sequence are produced. Thus, primers initiate amplification of the target sequence when it is present under amplification conditions.

Generally, two primers which are complementary to a portion of a target strand and its complement are employed in PCR. For LCR, four probes, two of which are complementary to a target sequence and two of which are similarly complementary to the targets complement, are generally employed. In addition to the primer sets and enzymes previously mentioned, a nucleic acid amplification reaction mixture may also comprise other reagents which are well known and include but are

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not limited to: enzyme cofactors such as manganese; magnesium; salts; nicotinamide adenine dinucleotide (NAD); and deoxynucleotide triphosphates (dNTPs) such as for example deoxyadenine triphosphate, deoxyguanine triphosphate, deoxycytosine triphosphate and deoxythymine triphosphate.

5           While the amplification primers initiate amplification of the target sequence, the detection (or hybridization) probe is not involved in amplification. Detection probes are generally nucleic acid sequences or uncharged nucleic acid analogs such as, for example, peptide nucleic acids which are disclosed in International Publication No. WO 92/20702; morpholino analogs which are described in U.S.  
10   Patents Nos 5,185,444, 5,034,506 and 5,142,047; and the like. Depending upon the type of label carried by the probe, the probe is employed to capture or detect the amplicon generated by the amplification reaction. The probe is not involved in amplification of the target sequence and therefore may have to be rendered "non-extendible" in that additional dNTPs cannot be added to the probe. In and of  
15   themselves analogs usually are non-extendible and nucleic acid probes can be rendered non-extendible by modifying the 3' end of the probe such that the hydroxyl group is no longer capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection label to thereby  
20   consume or otherwise block the hydroxyl group. Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified. U.S. Patent Application Serial No. 07/049,061 filed April 19, 1993 and incorporated herein by reference describes modifications which can be used to render a probe non-extendible.

          Accordingly, the ratio of primers to probes is not important. Thus, either the probes or primers can be added to the reaction mixture in excess whereby the  
25   concentration of one would be greater than the concentration of the other. Alternatively, primers and probes can be employed in equivalent concentrations. Preferably, however, the primers are added to the reaction mixture in excess of the probes. Thus, primer to probe ratios of, for example, 5:1 and 20:1 are preferred.

          While the length of the primers and probes can vary, the probe sequences are  
30   selected such that they have a lower melt temperature than the primer sequences. Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the range of between 20 and 50 nucleotides long, more typically in the range of between 20 and 30 nucleotides long. The typical probe is in the range of between 10 and 25 nucleotides long.

35           Various methods for synthesizing primers and probes are well known in the art. Similarly, methods for attaching labels to primers or probes are also well known

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in the art. For example, it is a matter of routine to synthesize desired nucleic acid primers or probes using conventional nucleotide phosphoramidite chemistry and instruments available from Applied Biosystems, Inc., (Foster City, CA), DuPont (Wilmington, DE), or Milligen (Bedford MA). Many methods have been described for labeling oligonucleotides such as the primers or probes of the present invention. Enzo Biochemical (New York, NY) and Clontech (Palo Alto, CA) both have described and commercialized probe labeling techniques. For example, a primary amine can be attached to a 3' oligo terminus using 3'-Amine-ON CPG™ (Clontech, Palo Alto, CA). Similarly, a primary amine can be attached to a 5' oligo terminus using Aminomodifier II® (Clontech). The amines can be reacted to various haptens using conventional activation and linking chemistries. In addition, copending applications U.S. Serial Nos. 625,566, filed December 11, 1990 and 630,908, filed December 20, 1990, which are each incorporated herein by reference, teach methods for labeling probes at their 5' and 3' termini, respectively. International Publication Nos WO 92/10505, published 25 June 1992, and WO 92/11388, published 9 July 1992, teach methods for labeling probes at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. See, for example, N.T. Thuong et al., Tet. Letters 29(46):5905-5908 (1988); or J.S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' and 5' ends.

Capture labels are attached to the primers or probes and can be a specific binding member which forms a binding pair with the solid phase reagent's specific binding member. It will be understood that the primer or probe itself may serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of the primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where the probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the single stranded amplicon members. In the case where the primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase because the probe is selected such that it is not fully complementary to the primer sequence.

Generally, probe/single stranded amplicon member complexes can be detected using techniques commonly employed to perform heterogeneous



immunoassays. Preferably, in this embodiment, detection is performed according to the protocols used by the commercially available Abbott LCx<sup>®</sup> instrumentation (Abbott Laboratories, Abbott Park, IL).

5 The primers and probes disclosed herein are useful in typical PCR assays, wherein the test sample is contacted with a pair of primers, amplification is performed, the hybridization probe is added, and detection is performed.

Another method provided by the present invention comprises contacting a test sample with a plurality of polynucleotides, wherein at least one polynucleotide is a BS203 molecule as described herein, hybridizing the test sample with the plurality of polynucleotides and detecting hybridization complexes. Hybridization complexes are identified and quantitated to compile a profile which is indicative breast tissue disease, such as breast cancer. Expressed RNA sequences may further be detected by reverse transcription and amplification of the DNA product by procedures well-known in the art, including polymerase chain reaction (PCR).

15 Drug Screening and Gene Therapy.

The present invention also encompasses the use of gene therapy methods for the introduction of anti-sense BS203 derived molecules, such as polynucleotides or oligonucleotides of the present invention, into patients with conditions associated with abnormal expression of polynucleotides related to a breast tissue disease or condition especially breast cancer. These molecules, including antisense RNA and DNA fragments and ribozymes, are designed to inhibit the translation of BS203-mRNA, and may be used therapeutically in the treatment of conditions associated with altered or abnormal expression of BS203 polynucleotide.

25 Alternatively, the oligonucleotides described above can be delivered to cells by procedures known in the art such that the anti-sense RNA or DNA may be expressed in vivo to inhibit production of a BS203 polypeptide in the manner described above. Antisense constructs to BS203 polynucleotide, therefore, reverse the action of BS203 transcripts and may be used for treating breast tissue disease conditions, such as breast cancer. These antisense constructs may also be used to treat tumor metastases.

30 The present invention also provides a method of screening a plurality of compounds for specific binding to BS203 polypeptide(s), or any fragment thereof, to identify at least one compound which specifically binds the BS203 polypeptide. Such a method comprises the steps of providing at least one compound; combining the BS203 polypeptide with each compound under suitable conditions for a time

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sufficient to allow binding; and detecting the BS203 polypeptide binding to each compound.

5 The polypeptide or peptide fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transfected with recombinant nucleic acids which can express the polypeptide or peptide fragment. Drugs may be screened against such transfected cells in competitive binding assays. For example, the formation of complexes between a polypeptide and the agent being tested can be measured in  
10 either viable or fixed cells.

The present invention thus provides methods of screening for drugs or any other agent which can be used to treat diseases associated with BS203. These methods comprise contacting the drug with a polypeptide or fragment thereof and assaying for either the presence of a complex between the agent and the polypeptide,  
15 or for the presence of a complex between the polypeptide and the cell. In competitive binding assays, the polypeptide typically is labeled. After suitable incubation, free (or uncomplexed) polypeptide or fragment thereof is separated from that present in bound form, and the amount of free or uncomplexed label is used as a measure of the ability of the particular drug to bind to polypeptide or to interfere with  
20 the polypeptide/cell complex.

The present invention also encompasses the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptide specifically compete with a test drug for binding to the polypeptide or fragment thereof. In this manner, the antibodies can be used to detect the presence of any  
25 polypeptide in the test sample which shares one or more antigenic determinants with a BS203 polypeptide as provided herein.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to at least one polypeptide of BS203 disclosed herein. Briefly, large numbers of different small peptide test compounds  
30 are synthesized on a solid phase, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptide and washed. Polypeptide thus bound to the solid phase is detected by methods well-known in the art. Purified polypeptide can also be coated directly onto plates for use in the drug screening techniques described herein. In addition, non-neutralizing antibodies can be used to  
35 capture the polypeptide and immobilize it on the solid support. See, for example, EP

84/03564, published on September 13, 1984, which is incorporated herein by reference.

5       The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of the small molecules including agonists, antagonists, or inhibitors with which they interact. Such structural analogs can be used to design drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide in vivo. J. Hodgson, Bio/Technology 9:19-21 (1991), incorporated herein by reference.

10       For example, in one approach, the three-dimensional structure of a polypeptide, or of a polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide may be gained by  
15       modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous polypeptide-like molecules or to identify efficient inhibitors

20       Useful examples of rational drug design may include molecules which have improved activity or stability as shown by S. Braxton et al., Biochemistry 31:7796-7801 (1992), or which act as inhibitors, agonists, or antagonists of native peptides as shown by S.B.P. Athauda et al., J Biochem. (Tokyo) 113 (6):742-746 (1993), incorporated herein by reference.

25       It also is possible to isolate a target-specific antibody selected by an assay as described hereinabove, and then to determine its crystal structure. In principle this approach yields a pharmacophore upon which subsequent drug design can be based. It further is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies ("anti-ids") to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-id is an analog of the original receptor. The anti-id then can be used to identify and isolate peptides from  
30       banks of chemically or biologically produced peptides. The isolated peptides then can act as the pharmacophore (that is, a prototype pharmaceutical drug).

35       A sufficient amount of a recombinant polypeptide of the present invention may be made available to perform analytical studies such as X-ray crystallography. In addition, knowledge of the polypeptide amino acid sequence which are derivable from the nucleic acid sequence provided herein will provide guidance to those

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employing computer modeling techniques in place of, or in addition to, x-ray crystallography.

Antibodies specific to a BS203 polypeptide (e.g., anti-BS203 antibodies) further may be used to inhibit the biological action of the polypeptide by binding to the polypeptide. In this manner, the antibodies may be used in therapy, for example, to treat breast tissue diseases including breast cancer and its metastases.

Further, such antibodies can detect the presence or absence of BS203 polypeptide in a test sample and, therefore, are useful as diagnostic markers for the diagnosis of a breast tissue disease or condition especially breast cancer. Such antibodies may also function as a diagnostic marker for breast tissue disease conditions such as breast cancer. The present invention also is directed to antagonists and inhibitors of the polypeptides of the present invention. The antagonists and inhibitors are those which inhibit or eliminate the function of the polypeptide. Thus, for example, an antagonist may bind to a polypeptide of the present invention and inhibit or eliminate its function. The antagonist, for example, could be an antibody against the polypeptide which eliminates the activity of BS203 polypeptide by binding BS203 polypeptide, or in some cases the antagonist may be an oligonucleotide. Examples of small molecule inhibitors include but are not limited to small peptides or peptide-like molecules.

The antagonists and inhibitors may be employed as a composition with a pharmaceutically acceptable carrier, including but not limited to saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. Administration of BS203 polypeptide inhibitors is preferably systemic. The present invention also provides an antibody which inhibits the action of such polypeptide.

Antisense technology can be used to reduce gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the polypeptide of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription, thereby preventing transcription and the production of the BS203 polypeptide. For triple helix, see, for example, Lee et al, Nuc. Acids Res. 6:3073 (1979); Cooney et al, Science 241:456 (1988); and Dervan et al, Science 251:1360 (1991) The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of an mRNA molecule into the BS203 polypeptide. For antisense, see, for example, Okano, J. Neurochem. 56:560

(1991); and "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression," CRC Press, Boca Raton, Fla. (1988). Antisense oligonucleotides act with greater efficacy when modified to contain artificial internucleotide linkages which render the molecule resistant to nucleolytic cleavage. Such artificial internucleotide linkages include but are not limited to methylphosphonate, phosphorothiolate and phosphoroamidate internucleotide linkages.

Recombinant Technology.

The present invention provides host cells and expression vectors comprising BS203 polynucleotides of the present invention and methods for the production of the polypeptide(s) they encode. Such methods comprise culturing the host cells under conditions suitable for the expression of the BS203 polynucleotide and recovering the BS203 polypeptide from the cell culture.

The present invention also provides vectors which include BS203 polynucleotides of the present invention, host cells which are genetically engineered with vectors of the present invention and the production of polypeptides of the present invention by recombinant techniques.

Host cells are genetically engineered (transfected, transduced or transformed) with the vectors of this invention which may be a cloning vector or an expression vector. The vector may be in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transfected cells, or amplifying BS203 gene(s). The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing a polypeptide by recombinant techniques. Thus, the polynucleotide sequence may be included in any one of a variety of expression vehicles, in particular vectors or plasmids for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus and pseudorabies. However, any other plasmid or vector may be used so long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into appropriate restriction endonuclease sites by procedures known in the art. Such procedures and others are

deemed to be within the scope of those skilled in the art. The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. Representative examples of such promoters include but are not limited to LTR or SV40 promoter, the E. coli lac or trp, the phage lambda P sub L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of transfected host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transfect an appropriate host to permit the host to express the protein. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Salmonella typhimurium; Streptomyces sp.; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings provided herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available. The following vectors are provided by way of example. Bacterial: pINCY (Incyte Pharmaceuticals Inc., Palo Alto, CA), pSPORT1 (Life Technologies, Gaithersburg, MD), pQE70, pQE60, pQE-9 (Qiagen) pBs, phagescript, psiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

Plasmid pINCY is generally identical to the plasmid pSPORT1 (available from Life Technologies, Gaithersburg, MD) with the exception that it has two modifications in the polylinker (multiple cloning site). These modifications are (1) it lacks a HindIII restriction site and (2) its EcoRI restriction site lies at a different location. pINCY is created from pSPORT1 by cleaving pSPORT1 with both HindIII and EcoRI and replacing the excised fragment of the polylinker with synthetic DNA fragments (SEQUENCE ID NO 15 and SEQUENCE ID NO 16). This replacement may be made in any manner known to those of ordinary skill in the art. For example, the two nucleotide sequences, SEQUENCE ID NO 15 and SEQUENCE ID NO 16, may be generated synthetically with 5' terminal phosphates, mixed together and then ligated under standard conditions for performing staggered end ligations into the pSPORT1 plasmid cut with HindIII and EcoRI. Suitable host cells (such as *E. coli* DH5 $\alpha$  cells) then are transfected with the ligated DNA and recombinant clones are selected for ampicillin resistance. Plasmid DNA then is prepared from individual clones and subjected to restriction enzyme analysis or DNA sequencing in order to confirm the presence of insert sequences in the proper orientation. Other cloning strategies known to the ordinary artisan also may be employed.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, SP6, T7, gpt, lambda P sub R, P sub L and trp. Eukaryotic promoters include cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, LTRs from retroviruses and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention provides host cells containing the above-described construct. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (L. Davis et al., "Basic Methods in Molecular Biology," 2nd edition, Appleton and Lang, Paramount Publishing, East Norwalk, CT (1994).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the

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polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Recombinant proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor, N.Y., 1989), which is hereby incorporated by reference.

Transcription of a DNA encoding the polypeptide(s) of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin (bp 100 to 270), a cytomegalovirus early promoter enhancer, a polyoma enhancer on the late side of the replication origin and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transfection of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), alpha factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable marker s and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transfection include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within



the genera Pseudomonas, Streptomyces and Staphylococcus, although others may also be employed as a routine matter of choice.

Useful expression vectors for bacterial use comprise a selectable marker and bacterial origin of replication derived from plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Other vectors include but are not limited to PKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transfection of a suitable host and growth of the host to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction), and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents; such methods are well-known to the ordinary artisan.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, such as the C127, HEK-293, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Representative, useful vectors include pRc/CMV and pcDNA3 (available from Invitrogen, San Diego, CA).

BS203 polypeptides are recovered and purified from recombinant cell cultures by known methods including affinity chromatography, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography or lectin chromatography. It is preferred to have low concentrations (approximately 0.1-5 mM) of calcium ion present during purification (Price, et al., J. Biol. Chem. 244:917 [1969]). Protein refolding steps

can be used, as necessary, in completing configuration of the polypeptide. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Thus, polypeptides of the present invention may be naturally purified products expressed from a high expressing cell line, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. The polypeptides of the invention may also include an initial methionine amino acid residue.

The starting plasmids can be constructed from available plasmids in accord with published, known procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

The following is the general procedure for the isolation and analysis of cDNA clones. In a particular embodiment disclosed herein, mRNA was isolated from breast tissue and used to generate the cDNA library. Breast tissue was obtained from patients by surgical resection and was classified as tumor or non-tumor tissue by a pathologist.

The cDNA inserts from random isolates of the breast tissue libraries were sequenced in part, analyzed in detail as set forth in the Examples and are disclosed in the Sequence Listing as SEQUENCE ID NOS 1-13. The consensus sequence of these inserts is presented as SEQUENCE ID NO 14. These polynucleotides may contain an entire open reading frame with or without associated regulatory sequences for a particular gene, or they may encode only a portion of the gene of interest. This is attributed to the fact that many genes are several hundred and sometimes several thousand, bases in length and, with current technology, cannot be cloned in their entirety because of vector limitations, incomplete reverse transcription of the first strand, or incomplete replication of the second strand. Contiguous, secondary clones containing additional nucleotide sequence may be obtained using a variety of methods known to those of skill in the art.

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ DNA polymerase, Klenow fragment, Sequenase (US Biochemical Corp, Cleveland, OH) or Taq polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have

been developed for the use of both single-stranded and double-stranded templates. The chain termination reaction products may be electrophoresed on urea/polyacrylamide gels and detected either by autoradiography (for radionucleotide labeled precursors) or by fluorescence (for fluorescent-labeled precursors). Recent  
5 improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day using machines such as the Applied Biosystems 377 DNA Sequencers (Applied Biosystems, Foster City, CA).

The reading frame of the nucleotide sequence can be ascertained by several  
10 types of analyses. First, reading frames contained within the coding sequence can be analyzed for the presence of start codon ATG and stop codons TGA, TAA or TAG. Typically, one reading frame will continue throughout the major portion of a cDNA sequence while other reading frames tend to contain numerous stop codons. In such cases reading frame determination is straightforward. In other more difficult  
15 cases, further analysis is required.

Algorithms have been created to analyze the occurrence of individual nucleotide bases at each putative codon triplet. See, for example J.W. Fickett, Nuc Acids Res 10:5303 (1982). Coding DNA for particular organisms (bacteria, plants and animals) tends to contain certain nucleotides within certain triplet periodicities,  
20 such as a significant preference for pyrimidines in the third codon position. These preferences have been incorporated into widely available software which can be used to determine coding potential (and frame) of a given stretch of DNA. The algorithm-derived information combined with start/stop codon information can be used to determine proper frame with a high degree of certainty. This, in turn, readily  
25 permits cloning of the sequence in the correct reading frame into appropriate expression vectors.

The nucleic acid sequences disclosed herein may be joined to a variety of other polynucleotide sequences and vectors of interest by means of well established recombinant DNA techniques. See J. Sambrook et al., supra. Vectors of interest  
30 include cloning vectors, such as plasmids, cosmids, phage derivatives, phagemids, as well as sequencing, replication and expression vectors, and the like. In general, such vectors contain an origin of replication functional in at least one organism, convenient restriction endonuclease digestion sites and selectable markers appropriate for particular host cells. The vectors can be transferred by a variety of  
35 means known to those of skill in the art into suitable host cells which then produce the desired DNA, RNA or polypeptides.

Occasionally, sequencing or random reverse transcription errors will mask the presence of the appropriate open reading frame or regulatory element. In such cases, it is possible to determine the correct reading frame by attempting to express the polypeptide and determining the amino acid sequence by standard peptide mapping and sequencing techniques. See, F.M. Ausubel, et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY (1989). Additionally, the actual reading frame of a given nucleotide sequence may be determined by transfection of host cells with vectors containing all three potential reading frames. Only those cells with the nucleotide sequence in the correct reading frame will produce a peptide of the predicted length.

The nucleotide sequences provided herein have been prepared by current, state-of-the-art, automated methods and as such may contain unidentified nucleotides. These will not present a problem to those skilled in the art who wish to practice the invention. Several methods employing standard recombinant techniques, described in J. Sambrook (supra) or periodic updates thereof, may be used to complete the missing sequence information. The same techniques used for obtaining a full length sequence, as described herein, may be used to obtain nucleotide sequence.

Expression of a particular cDNA may be accomplished by subcloning the cDNA into an appropriate expression vector and transfecting this vector into an appropriate expression host. The cloning vector used for the generation of the breast tissue cDNA library can be used for transcribing mRNA of a particular cDNA and contains a promoter for beta-galactosidase, an amino-terminal met and the subsequent seven amino acid residues of beta-galactosidase. Immediately following these eight residues is an engineered bacteriophage promoter useful for artificial priming and transcription and a number of unique restriction sites, including EcoRI, for cloning. The vector can be transfected into an appropriate host strain of E. coli.

Induction of the isolated bacterial strain with isopropylthiogalactoside (IPTG) using standard methods will produce a fusion protein which contains the first seven residues of beta-galactosidase, about 15 residues of linker and the peptide encoded within the cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, the correct frame can be obtained by deletion or insertion of an appropriate number of bases by well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

5 The cDNA can be shuttled into other vectors known to be useful for  
expression of protein in specific hosts. Oligonucleotide primers containing cloning  
sites and segments of DNA sufficient to hybridize to stretches at both ends of the  
target cDNA can be synthesized chemically by standard methods. These primers can  
then be used to amplify the desired gene segments by PCR. The resulting new gene  
segments can be digested with appropriate restriction enzymes under standard  
conditions and isolated by gel electrophoresis. Alternately, similar gene segments  
can be produced by digestion of the cDNA with appropriate restriction enzymes and  
filling in the missing gene segments with chemically synthesized oligonucleotides.  
10 Segments of the coding sequence from more than one gene can be ligated together  
and cloned in appropriate vectors to optimize expression of recombinant sequence.

Suitable expression hosts for such chimeric molecules include but are not  
limited to, mammalian cells such as Chinese Hamster Ovary (CHO) and human  
embryonic kidney (HEK) 293 cells, insect cells such as Sf9 cells, yeast cells such as  
15 Saccharomyces cerevisiae and bacteria such as E. coli. For each of these cell  
systems, a useful expression vector may also include an origin of replication to allow  
propagation in bacteria and a selectable marker such as the beta-lactamase antibiotic  
resistance gene to allow selection in bacteria. In addition, the vectors may include a  
second selectable marker such as the neomycin phosphotransferase gene to allow  
20 selection in transfected eukaryotic host cells. Vectors for use in eukaryotic  
expression hosts may require the addition of 3' poly A tail if the sequence of interest  
lacks poly A.

Additionally, the vector may contain promoters or enhancers which increase  
gene expression. Such promoters are host specific and include but are not limited to  
25 MMTV, SV40, or metallothionine promoters for CHO cells; trp, lac, tac or T7  
promoters for bacterial hosts; or alpha factor, alcohol oxidase or PGH promoters for  
yeast. Adenoviral vectors with or without transcription enhancers, such as the rous  
sarcoma virus (RSV) enhancer, may be used to drive protein expression in  
mammalian cell lines. Once homogeneous cultures of recombinant cells are  
30 obtained, large quantities of recombinantly produced protein can be recovered from  
the conditioned medium and analyzed using chromatographic methods well known  
in the art. An alternative method for the production of large amounts of secreted  
protein involves the transfection of mammalian embryos and the recovery of the  
recombinant protein from milk produced by transgenic cows, goats, sheep, etc.  
35 Polypeptides and closely related molecules may be expressed recombinantly in such  
a way as to facilitate protein purification. One approach involves expression of a

chimeric protein which includes one or more additional polypeptide domains not naturally present on human polypeptides. Such purification-facilitating domains include, but are not limited to, metal-chelating peptides such as histidine-tryptophan domains that allow purification on immobilized metals, protein A domains that allow  
5 purification on immobilized immunoglobulin and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase from Invitrogen (San Diego, CA) between the polypeptide sequence and the purification domain may be useful for recovering the polypeptide.

10       Immunoassays.

BS203 polypeptides, including fragments, derivatives, and analogs thereof, or cells expressing such polypeptides, can be utilized in a variety of assays, many of which are described herein, for the detection of antibodies to breast tissue. They also can be used as immunogens to produce antibodies. These antibodies can be, for  
15 example, polyclonal or monoclonal antibodies, chimeric, single chain and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

For example, antibodies generated against a polypeptide comprising a  
20 sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal such as a mouse, rabbit, goat or human. A mouse, rabbit or goat is preferred. The polypeptide is selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20,  
25 SEQUENCE ID NO 21, and fragments thereof. The antibody so obtained then will bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies that bind the native polypeptide. Such antibodies then can be used to isolate the polypeptide from test samples such as tissue suspected of containing that polypeptide. For preparation of  
30 monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique as described by Kohler and Milstein, Nature 256:495-497 (1975), the trioma technique, the human B-cell hybridoma technique as described by Kozbor et al, Immun. Today 4:72 (1983) and the EBV-hybridoma technique to produce human  
35 monoclonal antibodies as described by Cole, et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc, New York, NY, pp. 77-96 (1985). Techniques

described for the production of single chain antibodies can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. See, for example, U.S. Patent No. 4,946,778, which is incorporated herein by reference.

Various assay formats may utilize the antibodies of the present invention, including "sandwich" immunoassays and probe assays. For example, the antibodies of the present invention, or fragments thereof, can be employed in various assay systems to determine the presence, if any, of BS203 antigen in a test sample. For example, in a first assay format, a polyclonal or monoclonal antibody or fragment thereof, or a combination of these antibodies, which has been coated on a solid phase, is contacted with a test sample, to form a first mixture. This first mixture is incubated for a time and under conditions sufficient to form antigen/antibody complexes. Then, an indicator reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, or a combination of these antibodies, to which a signal generating compound has been attached, is contacted with the antigen/antibody complexes to form a second mixture. This second mixture then is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence of BS203 antigen in the test sample and captured on the solid phase, if any, is determined by detecting the measurable signal generated by the signal generating compound. The amount of BS203 antigen present in the test sample is proportional to the signal generated.

In an alternative assay format, a mixture is formed by contacting: (1) a polyclonal antibody, monoclonal antibody, or fragment thereof, which specifically binds to BS203 antigen, or a combination of such antibodies bound to a solid support; (2) the test sample; and (3) an indicator reagent comprising a monoclonal antibody, polyclonal antibody, or fragment thereof, which specifically binds to a different BS203 antigen (or a combination of these antibodies) to which a signal generating compound is attached. This mixture is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence, if any, of BS203 antigen present in the test sample and captured on the solid phase is determined by detecting the measurable signal generated by the signal generating compound. The amount of BS203 antigen present in the test sample is proportional to the signal generated.

In another assay format, one or a combination of at least two monoclonal antibodies of the invention can be employed as a competitive probe for the detection of antibodies to BS203 antigen. For example, BS203 polypeptides such as the recombinant antigens disclosed herein, either alone or in combination, are coated on

a solid phase. A test sample suspected of containing antibody to BS203 antigen then is incubated with an indicator reagent comprising a signal generating compound and at least one monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent bound to the solid phase or the indicator reagent bound to the solid phase. The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively measured.

In yet another detection method, each of the monoclonal or polyclonal antibodies of the present invention can be employed in the detection of BS203 antigens in tissue sections, as well as in cells, by immunohistochemical analysis. Cytochemical analysis wherein these antibodies are labeled directly (with, for example, fluorescein, colloidal gold, horseradish peroxidase, alkaline phosphatase, etc.) or are labeled by using secondary labeled anti-species antibodies (with various labels as exemplified herein) to track the histopathology of disease also are within the scope of the present invention.

In addition, these monoclonal antibodies can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific BS203 polypeptides from cell cultures or biological tissues such as to purify recombinant and native BS203 proteins.

The monoclonal antibodies of the invention also can be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

The monoclonal antibodies or fragments thereof can be provided individually to detect BS203 antigens. Combinations of the monoclonal antibodies (and fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least one BS203 antibody of the invention, along with antibodies which specifically bind to other BS203 regions, each antibody having different binding specificities. Thus, this cocktail can include the monoclonal antibodies of the invention which are directed to BS203 polypeptides disclosed herein and other monoclonal antibodies specific to other antigenic determinants of BS203 antigens or other related proteins.

The polyclonal antibody or fragment thereof which can be used in the assay formats should specifically bind to a BS203 polypeptide or other BS203 polypeptides additionally used in the assay. The polyclonal antibody used preferably is of mammalian origin such as, human, goat, rabbit or sheep polyclonal antibody which binds BS203 polypeptide. Most preferably, the polyclonal antibody is of rabbit origin. The polyclonal antibodies used in the assays can be used either



alone or as a cocktail of polyclonal antibodies. Since the cocktails used in the assay formats are comprised of either monoclonal antibodies or polyclonal antibodies having different binding specificity to BS203 polypeptides, they are useful for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating,  
5 or determining the predisposition to, diseases and conditions of the breast such as breast cancer.

It is contemplated and within the scope of the present invention that BS203 antigen may be detectable in assays by use of a recombinant antigen as well as by use of a synthetic peptide or purified peptide, which peptide comprises an amino acid  
10 sequence of BS203. The amino acid sequence of such a polypeptide is selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof. It also is within the scope of the present invention that different synthetic, recombinant or purified peptides identifying different epitopes of BS203,  
15 can be used in combination in an assay for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to diseases and conditions of the breast such as breast cancer. In this case, all of these peptides can be coated onto one solid phase; or each separate peptide may be coated onto separate solid phases, such as microparticles, and then  
20 combined to form a mixture of peptides which can be later used in assays. Furthermore, it is contemplated that multiple peptides which define epitopes from different antigens may be used for the detection, diagnosis, staging, monitoring, prognosis, prevention or treatment of, or determining the predisposition to, diseases and conditions of the breast, such as breast cancer. Peptides coated on solid phases  
25 or labeled with detectable labels are then allowed to compete with those present in a patient sample (if any) for a limited amount of antibody. A reduction in binding of the synthetic, recombinant, or purified peptides to the antibody (or antibodies) is an indication of the presence of BS203 antigen in the patient sample. The presence of BS203 antigen indicates the presence of breast tissue disease, especially breast  
30 cancer, in the patient. Variations of assay formats are known to those of ordinary skill in the art and many are discussed herein below.

In another assay format, the presence of anti-BS203 antibody and/or BS203 antigen can be detected in a simultaneous assay, as follows. A test sample is simultaneously contacted with a capture reagent of a first analyte, wherein said  
35 capture reagent comprises a first binding member specific for a first analyte attached to a solid phase and a capture reagent for a second analyte, wherein said capture

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reagent comprises a first binding member for a second analyte attached to a second solid phase, to thereby form a mixture. This mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte and capture reagent/second analyte complexes. These so-formed complexes then are contacted  
5 with an indicator reagent comprising a member of a binding pair specific for the first analyte labeled with a signal generating compound and an indicator reagent comprising a member of a binding pair specific for the second analyte labeled with a signal generating compound to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form capture reagent/first  
10 analyte/indicator reagent complexes and capture reagent/second analyte/indicator reagent complexes. The presence of one or more analytes is determined by detecting a signal generated in connection with the complexes formed on either or both solid phases as an indication of the presence of one or more analytes in the test sample. In this assay format, recombinant antigens derived from the expression systems  
15 disclosed herein may be utilized, as well as monoclonal antibodies produced from the proteins derived from the expression systems as disclosed herein. For example, in this assay system BS203 antigen can be the first analyte. Such assay systems are described in greater detail in EP Publication No. 0473065.

In yet other assay formats, the polypeptides disclosed herein may be utilized  
20 to detect the presence of antibody against BS203 antigen in test samples. For example, a test sample is incubated with a solid phase to which at least one polypeptide such as a recombinant protein or synthetic peptide has been attached. The polypeptide is selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20,  
25 SEQUENCE ID NO 21, and fragments thereof. These are reacted for a time and under conditions sufficient to form antigen/antibody complexes. Following incubation, the antigen/antibody complex is detected. Indicator reagents may be used to facilitate detection, depending upon the assay system chosen. In another assay format, a test sample is contacted with a solid phase to which a recombinant  
30 protein produced as described herein is attached, and also is contacted with a monoclonal or polyclonal antibody specific for the protein, which preferably has been labeled with an indicator reagent. After incubation for a time and under conditions sufficient for antibody/antigen complexes to form, the solid phase is separated from the free phase, and the label is detected in either the solid or free  
35 phase as an indication of the presence of antibody against BS203 antigen. Other assay formats utilizing the recombinant antigens disclosed herein are contemplated.

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These include contacting a test sample with a solid phase to which at least one antigen from a first source has been attached, incubating the solid phase and test sample for a time and under conditions sufficient to form antigen/antibody complexes, and then contacting the solid phase with a labeled antigen, which antigen is derived from a second source different from the first source. For example, a recombinant protein derived from a first source such as E. coli is used as a capture antigen on a solid phase, a test sample is added to the so-prepared solid phase, and following standard incubation and washing steps as deemed or required, a recombinant protein derived from a different source (i.e., non-E. coli) is utilized as a part of an indicator reagent which subsequently is detected. Likewise, combinations of a recombinant antigen on a solid phase and synthetic peptide in the indicator phase also are possible. Any assay format which utilizes an antigen specific for BS203 produced or derived from a first source as the capture antigen and an antigen specific for BS203 from a different second source are contemplated. Thus, various combinations of recombinant antigens, as well as the use of synthetic peptides, purified proteins and the like, are within the scope of this invention. Assays such as this and others are described in U.S. Patent No. 5,254,458, which enjoys common ownership and is incorporated herein by reference.

Other embodiments which utilize various other solid phases also are contemplated and are within the scope of this invention. For example, ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer (described in EP publication 0326100 and EP publication No. 0406473), can be employed according to the present invention to effect a fast solution-phase immunochemical reaction. An immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged poly-anion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems previously described, including those described in chemiluminescent signal measurements as described in EPO Publication No. 0 273,115.

Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including automated and semi-automated systems wherein the solid phase comprises a microparticle (magnetic or non-magnetic). Such systems include those described, in for example, published EPO applications Nos. EP 0 425 633 and EP 0 424 634, respectively.

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies of the present invention are easily

adaptable. In scanning probe microscopy, particularly in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the invention, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunneling microscopy eliminates the need for labels which normally must be utilized in many immunoassay systems to detect antigen/antibody complexes. The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (analyte specific substance which is the monoclonal antibody of the invention) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test piece which comprises a solid phase of a plastic or metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Also, polyelectrolyte interactions may be used to immobilize a specific binding partner on a surface of a test piece by using techniques and chemistries. The preferred method of attachment is by covalent means. Following attachment of a specific binding member, the surface may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding. The surface also may be scanned either at the site of manufacture or point of use to verify its suitability for assay purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

While the present invention discloses the preference for the use of solid phases, it is contemplated that the reagents such as antibodies, proteins and peptides of the present invention can be utilized in non-solid phase assay systems. These assay systems are known to those skilled in the art, and are considered to be within the scope of the present invention.

It is contemplated that the reagent employed for the assay can be provided in the form of a test kit with one or more containers such as vials or bottles, with each container containing a separate reagent such as a probe, primer, monoclonal antibody or a cocktail of monoclonal antibodies, or a polypeptide (e.g. recombinantly, synthetically produced or purified) employed in the assay. The polypeptide is

selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20 ,SEQUENCE ID NO 21, and fragments thereof. Other components such as buffers, controls and the like, known to those of ordinary skill in art, may be included in such test kits. It also is contemplated to provide test kits which have means for collecting test samples comprising accessible body fluids, e.g., blood, urine, saliva and stool. Such tools useful for collection ("collection materials") include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. Test kits designed for the collection, stabilization and preservation of test specimens obtained by surgery or needle biopsy are also useful. It is contemplated that all kits may be configured in two components which can be provided separately; one component for collection and transport of the specimen and the other component for the analysis of the specimen. The collection component, for example, can be provided to the open market user while the components for analysis can be provided to others such as laboratory personnel for determination of the presence, absence or amount of analyte. Further, kits for the collection, stabilization and preservation of test specimens may be configured for use by untrained personnel and may be available in the open market for use at home with subsequent transportation to a laboratory for analysis of the test sample.

E. coli bacteria (clone 2269559) has been deposited at the American Type Culture Collection (A.T.C.C.), 12301 Parklawn Drive, Rockville, Maryland 20852, as of \_\_\_\_\_, under the terms of the Budapest Treaty and will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, or for the enforceable period of the U.S. patent, whichever is longer. The deposit and any other deposited material described herein are provided for convenience only, and are not required to practice the present invention in view of the teachings provided herein. The cDNA sequence in all of the deposited material is incorporated herein by reference. Clone 2269559 was accorded A.T.C.C. Deposit No. \_\_\_\_\_.

The present invention will now be described by way of examples, which are meant to illustrate, but not to limit, the scope of the present invention.

### EXAMPLES

#### Example 1: Identification of Breast Tissue Library BS203 Gene-Specific Clones

A. Library Comparison of Expressed Sequence Tags (ESTs) or Transcript Images. Partial sequences of cDNA clone inserts, so-called “expressed sequence tags” (ESTs), were derived from cDNA libraries made from breast tumor tissues, breast non-tumor tissues and numerous other tissues, both tumor and non-tumor and entered into a database (LIFESEQ™ database, available from Incyte Pharmaceuticals, Palo Alto, CA) as gene transcript images. See International Publication No. WO 95/20681. A transcript image is a listing which provides the abundance of ESTs in a given tissue and represents the activity of genes in the tissue. The transcript images then were evaluated to identify EST sequences that were representative primarily of the breast tissue libraries. These target clones then were ranked according to their abundance (occurrence) in the target libraries and their absence from background libraries. Higher abundance clones with low background occurrence were given higher study priority. ESTs corresponding to the consensus sequence of BS203 were found in 27.8% (5 of 18) of breast tissue libraries. ESTs corresponding to the consensus sequence SEQUENCE ID NO 14 (or fragments thereof) were found in only 3.4% (12 of 355) of the other, non-breast, libraries of the data base. Therefore, the consensus sequence or fragment thereof was found more than 22 times more often in breast than non-breast tissues. SEQUENCE ID NOS 1-13, corresponding to overlapping clones 2269559, 1664718, 2360586, 1436565, 2479125, 2247228, 2112334, 960106, 962045, 959580, 961381, 2517547, and 2124915, respectively, were identified for further study. These represented the minimum number of clones that were needed to form the contig and from which the consensus sequence provided herein (SEQUENCE ID NO 14) was derived.

B. Generation of a Consensus Sequence. The nucleotide sequences of clones 2269559 (SEQUENCE ID NO 1), 1664718 (SEQUENCE ID NO 2), 2360586 (SEQUENCE ID NO 3), 1436565 (SEQUENCE ID NO 4), 2479125 (SEQUENCE ID NO 5), 2247228 (SEQUENCE ID NO 6), 2112334 (SEQUENCE ID NO 7), 960106 (SEQUENCE ID NO 8), 962045 (SEQUENCE ID NO 9), 959580 (SEQUENCE ID NO 10), 961381 (SEQUENCE ID NO 11), 2517547 (SEQUENCE ID NO 12), and 2124915 (SEQUENCE ID NO 13) were entered in the Sequencher™ Program (available from Gene Codes Corporation, Ann Arbor, MI, in order to generate a nucleotide alignment (contig map) and then generate their consensus sequence (SEQUENCE ID NO 14). FIGURES 1A-1C show the

nucleotide sequence alignment of these clones and their resultant nucleotide consensus sequence (SEQUENCE ID NO 14). FIGURE 2 presents the contig map depicting the clones SEQUENCE ID NOS 1-13 forming overlapping regions of the BS203 gene and the resultant consensus nucleotide sequence (SEQUENCE ID NO 14) of these clones in a graphic display. Following this, a three-frame translation was performed on the consensus sequence (SEQUENCE ID NO 14). The third forward frame was found to have an open reading frame encoding a 340 residue amino acid sequence, which is presented as SEQUENCE ID NO 17.

10                   Example 2: Sequencing of BS203 EST-Specific Clones

DNA sequences for clones which comprise the most upstream and downstream ESTs of the BS203 gene contig are determined using dideoxy termination sequencing with either dye-labeled primers, dye terminators, or radiolabeled nucleotides, following known methods. See, for example, F. Sanger et al., Proc. Natl. Acad. Sci. U.S.A. 74:5463 (1977).

Because vectors such as pSPORT1 (Life Technologies, Gaithersburg, MD) and pINCY (available from Incyte Pharmaceuticals, Inc., Palo Alto, CA) contain universal priming sites just adjacent to the 3' and 5' ligation junctions of the inserts, the inserts are sequenced in both directions using universal primers. The sequencing reactions are run on a polyacrylamide denaturing gel and the sequences are determined by an Applied Biosystems 377 Sequencer (available from Applied Biosystems, Foster City, CA) or other sequencing apparatus.

Example 3: Nucleic Acid Preparation

25           A. RNA Extraction from Tissue. Total RNA is isolated from solid breast tissues or cells and from non-breast tissues. Various methods can be utilized including but not limited to a lithium chloride/urea technique, known in the art and described by N. Kato et al., J. Virology 61:2182-2191 (1987), RNAzol™ B (available from Tel-Test, Inc., Friendswood, TX) and others.

30           B. RNA Extraction from Blood Mononuclear Cells. Mononuclear cells are isolated from blood samples from patients by centrifugation using Ficoll-Hypaque as follows. A 10 ml volume of whole blood is mixed with an equal volume of RPMI Medium (Life Technologies, Gaithersburg, MD). This mixture is then underlayered with 10 ml of Ficoll-Hypaque (Pharmacia, Piscataway, NJ) and centrifuged for 30 minutes at 200 x g. The buffy coat containing the mononuclear cells is removed, diluted to 50 ml with Dulbecco's PBS (Life Technologies, Gaithersburg, MD) and

the mixture centrifuged for 10 minutes at 200 x g. After two washes, the resulting pellet is resuspended in Dulbecco's PBS to a final volume of 1 ml.

RNA is prepared from the isolated mononuclear cells as described by N. Kato et al., J. Virology 61: 2182-2191 (1987). Briefly, the pelleted mononuclear cells are brought to a final of 1 ml volume and then are resuspended in 250  $\mu$ L of PBS and mixed with 2.5 ml of 3M LiCl, 6M urea, 5mM EDTA, 0.1M 2-mercaptoethanol, 50mM Tris-HCl (pH 7.5). The resulting mixture is homogenized and incubated at -20°C overnight. The homogenate is spun at 8,000 RPM in a Beckman J2-21M rotor for 90 minutes at 0-4°C. The pellet is resuspended in 10 ml 3M LiCl by vortexing and then spun at 10,000 RPM in a Beckman J2-21M rotor centrifuge for 45 minutes at 0-4°C. The resuspending and pelleting steps then are repeated. The pellet is resuspended in 2 ml of 1 mM EDTA, 0.5% SDS, 10 mM Tris (pH 7.5) and 400  $\mu$ g Proteinase K with vortexing and then it is incubated at 37°C for 30 minutes with shaking. One tenth volume of 3M NaCl then is added and the vortexed mixture. Proteins are removed by two cycles of extraction with phenol/ chloroform/ isoamyl alcohol followed by one extraction with chloroform/ isoamyl alcohol. RNA is precipitated by the addition of 6 ml of ethanol followed by overnight incubation at -20°C. After the precipitated RNA is collected by centrifugation, the pellet is washed 4 times in 75% ethanol. The pelleted RNA is then dissolved in 1mM EDTA, 10mM Tris-HCl (pH 7.5).

Non-breast tissues are used as negative controls. The mRNA can be further purified from total RNA by using commercially available kits such as oligo dT cellulose spin columns (RediCol™ from Pharmacia, Uppsala, Sweden) for the isolation of poly-adenylated RNA. Total or mRNA can be dissolved in lysis buffer (5M guanidine thiocyanate, 0.1M EDTA, pH 7.0) for analysis in the ribonuclease protection assay.

C. RNA Extraction from polysomes. Tissue is minced in saline at 4°C and mixed with 2.5 volumes of 0.8 M sucrose in a TK<sub>150</sub>M (150 mM KCl, 5 mM MgCl<sub>2</sub>, 50 mM Tris-HCl, pH 7.4) solution containing 6 mM 2-mercaptoethanol. The tissue is homogenized in a Teflon-glass Potter homogenizer with five strokes at 100-200 rpm followed by six strokes in a Dounce homogenizer, as described by B. Mechler, Methods in Enzymology 152:241-248 (1987). The homogenate then is centrifuged at 12,000 x g for 15 min at 4°C to sediment the nuclei. The polysomes are isolated by mixing 2 ml of the supernatant with 6 ml of 2.5 M sucrose in TK<sub>150</sub>M and layering this mixture over 4 ml of 2.5 M sucrose in TK<sub>150</sub>M in a 38 ml polyallomer tube. Two additional sucrose TK<sub>150</sub>M solutions are successively

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layered onto the extract fraction; a first layer of 13 ml 2.05 M sucrose followed by a second layer of 6 ml of 1.3 M sucrose. The polysomes are isolated by centrifuging the gradient at 90,000 x g for 5 h at 4°C. The fraction then is taken from the 1.3 M sucrose/2.05 M sucrose interface with a siliconized pasteur pipette and diluted in an equal volume of TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). An equal volume of 90°C SDS buffer (1% SDS, 200 mM NaCl, 20 mM Tris-HCl, pH 7.4) is added and the solution is incubated in a boiling water bath for 2 min. Proteins next are digested with a Proteinase-K digestion (50 mg/ml) for 15 min at 37°C. The mRNA is purified with 3 equal volumes of phenol-chloroform extractions followed by precipitation with 0.1 volume of 2 M sodium acetate (pH 5.2) and 2 volumes of 100% ethanol at -20°C overnight. The precipitated RNA is recovered by centrifugation at 12,000 x g for 10 min at 4°C. The RNA is dried and resuspended in TE (pH 7.4) or distilled water. The resuspended RNA then can be used in a slot blot or dot blot hybridization assay to check for the presence of BS203 mRNA (see Example 6).

The quality of nucleic acid and proteins is dependent on the method of preparation used. Each sample may require a different preparation technique to maximize isolation efficiency of the target molecule. These preparation techniques are within the skill of the ordinary artisan.

#### Example 4: Ribonuclease Protection Assay

##### A. Labeling of Complementary RNA (cRNA) Hybridization Probes.

Labeled sense and antisense riboprobes are transcribed from the BS203 gene cDNA sequence which contains a 5' RNA polymerase promoter such as SP6 or T7. The sequence may be from a vector containing the appropriate BS203 cDNA insert, or from a PCR-generated product of the insert using PCR primers which incorporate a 5' RNA polymerase promoter sequence. The transcripts are prepared in a 20 µl reaction volume containing 1 µg of DNA template, 2 µl of 100mM dithiothreitol, 0.8 µl of RNasin (10-40U), 500 µM each of ATP, CTP, GTP, 5 µl (alpha-<sup>32</sup>P) UTP or 100-500 µM biotinylated UTP and 1 µl of RNA polymerase in transcription buffer (40 mM Tris-HCl, pH 7.5, 6 mM MgCl<sub>2</sub>, 2 mM spermidine HCl, 5 mM NaCl). Following incubation at 37°C for one hour, the transcripts are treated with DNase I (15 U) for an additional 30 min to digest the template. The probes then are isolated by spin columns, salt precipitation or electrophoresis techniques which are well-known in the art. Finally, the probes are dissolved in lysis buffer (5 M Guanidine Thiocyanate, 0.1 M EDTA, pH 7.0).

5        B. Hybridization of Labeled Probe to Target. Approximately 20 µg of extracted total cellular RNA, as obtained in Example 3 supra, in 10 µl of lysis buffer are mixed with either (i)  $1 \times 10^5$  cpm of radioactively labeled probe or (ii) 250 pg of non-isotopically labeled probe, each in 2 µl of lysis buffer. The mixture then is incubated at 60°C for 5 min and hybridized overnight at room temperature. See, T. Kaabache et al., Anal. Biochem. 232:225-230 (1995).

10        C. RNase Digestion. Hybridizations are terminated by incubation with 380 µl of a solution containing 40 µg/ml RNase A and 625 units/ml RNase T1 in 1 mM EDTA, 300 mM NaCl, 30 mM Tris-HCl (pH 7.4) for 45-60 min at room temperature. RNase digestion then is terminated by the addition of 60 µl of Proteinase-K (1.7 mg/ml) containing 3.3% SDS, followed by incubation for 30 min at 37°C. The digested mixture then is extracted with phenol:chloroform:isoamyl alcohol to remove protein. The mRNA:cRNA hybrids are precipitated from the aqueous phase by the addition 4 µg yeast tRNA and 800 µl of ethanol and incubated at -80°C for 30 min. The precipitates are collected by centrifugation.

15        D Fragment Analysis. The precipitates are dissolved in 5 µl of denaturing gel loading dye (80% formamide, 10 mM EDTA, pH 8.0, 1 mg/ml xylene cyanol, 1 mg/ml bromophenol blue) and electrophoresed in 6% polyacrylamide TBE, 8 M urea denaturing gels. The gels are dried under vacuum and autoradiographed.

20        Quantitation can be performed by comparing the counts obtained from the test samples to a calibration curve that was generating by utilizing calibrators that are the sense strand. In cases where non-isotopic labels are used, hybrids are transferred from the gels to membranes (nylon or nitrocellulose) by blotting and then analyzed using detection systems that employ streptavidin alkaline phosphatase conjugates and chemiluminescence or chemifluorescence reagents. High level expression of mRNA corresponding to a sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof, then, is an indication of the presence of BS203 mRNA(s), suggesting a diagnosis of a breast tissue disease or condition, such as breast cancer.

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#### Example 5: Northern Blotting

The northern blot technique is used to identify a specific size RNA fragment from a complex population of RNA using gel electrophoresis and nucleic acid hybridization. Northern blotting is well-known technique in the art. Briefly, up to 20 µg of extracted RNA (see Example 3) is incubated in 20 µl of a solution containing 40 mM morpholinopropanesulfonic acid (MOPS), pH 7.0, 10 mM

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5 sodium acetate, 1 mM EDTA, 2.2 M formaldehyde, 50% v/v formamide for 15 min at 55°C. The denatured RNA is mixed with 2 µl of loading buffer (50% glycerol, 1 mM EDTA, 0.4% bromophenol blue, 0.4% xylene cyanol) and loaded into a denaturing 1.5% agarose gel containing 40 mM morpholinopropanesulfonic acid (MOPS), pH 7.0, 10 mM sodium acetate, 1 mM EDTA and 2.2 M formaldehyde. The gel is electrophoresed for an appropriate time, transferred to a wash tray and washed with five changes of RNase free water for 5 min followed by a 45 min soak at room temperature in 50 mM NaOH and 10 mM NaCl. The gel is neutralized by soaking for 45 min in 0.1 M Tris-HCl, pH 7.5. After a 1 h soak in 20X SSC buffer (3 M NaCl, 300 mM tri-sodium citrate), the gel is transferred onto a nitrocellulose or nylon based matrix. After transfer is complete, the filter is washed in 3X SSC, air dried for 2 h and baked at 80°C for 4 h under vacuum. The mRNAs are detected as in Example 4, supra. Again, high level of expression of mRNA corresponding to a sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof, is an indication of the presence of BS203 mRNA, suggesting a diagnosis of a breast tissue disease or condition, such as breast cancer.

Example 6: Dot Blot/Slot Blot

20 Dot and slot blot assays are quick methods to evaluate the presence of a specific nucleic acid sequence in a complex mix of nucleic acid. To perform such assays, up to 50 µg of RNA is mixed in 50 µl of 50% formamide, 7% formaldehyde, 1X SSC, incubated 15 min at 68°C, and then cooled on ice. Then, 100 µl of 20X SSC is added to the RNA mixture and loaded under vacuum onto a manifold apparatus that has a prepared nitrocellulose or nylon membrane. The membrane is soaked in water, 20X SSC for 1 hour, placed on two sheets of 20X SSC prewet Whatman #3 filter paper, and loaded into a slot blot or dot blot vacuum manifold apparatus. The slot blot is analyzed with probes prepared and labeled as described in Example 4, supra. Detection of mRNA corresponding to a sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof, is an indication of the presence of BS203, suggesting a diagnosis of a breast tissue disease or condition, such as breast cancer.

Other methods and buffers which can be utilized in the methods described in Examples 5 and 6, but not specifically detailed herein, are known in the art and are described in J. Sambrook et al, supra which is incorporated herein by reference.

Example 7: In Situ Hybridization

This method is useful to directly detect specific target nucleic acid sequences in cells using detectable nucleic acid hybridization probes.

Tissues are prepared with cross-linking fixative agents such as paraformaldehyde or glutaraldehyde for maximum cellular RNA retention. See, L. Angerer et al., Methods in Cell Biol. 35:37-71 (1991). Briefly, the tissue is placed in greater than 5 volumes of 1% glutaraldehyde in 50 mM sodium phosphate, pH 7.5 at 4°C for 30 min. The solution is changed with fresh glutaraldehyde solution (1% glutaraldehyde in 50mM sodium phosphate, pH 7.5) for a further 30 min fixing. The fixing solution should have an osmolality of approximately 0.375% NaCl. The tissue is washed once in isotonic NaCl to remove the phosphate.

The fixed tissues then are embedded in paraffin as follows. The tissue is dehydrated through a series of ethanol concentrations for 15 min each: 50% (twice), 70% (twice), 85%, 90% and then 100% (twice). Next, the tissue is soaked in two changes of xylene for 20 min each at room temperature. The tissue is then soaked in two changes of a 1:1 mixture of xylene and paraffin for 20 min each at 60°C; and then in three final changes of paraffin for 15 min each.

The tissue next is cut in 5 µm sections using a standard microtome and placed on a slide previously treated with a tissue adhesive such as 3-aminopropyltriethoxysilane.

Paraffin is removed from the tissue by two 10 min xylene soaks and rehydrated in a series of ethanol concentrations: 99% twice, 95%, 85%, 70%, 50%, 30%, and then distilled water twice. The sections are pre-treated with 0.2 M HCl for 10 min and permeabilized with 2 µg/ml Proteinase-K at 37°C for 15 min.

Labeled riboprobes transcribed from the BS203 gene plasmid (see Example 4) are hybridized to the prepared tissue sections and incubated overnight at 56°C in 3X standard saline extract and 50% formamide. Excess probe is removed by washing in 2X standard saline citrate and 50% formamide followed by digestion with 100 µg/ml RNase A at 37°C for 30 min. Fluorescence probe is visualized by illumination with ultraviolet (UV) light under a microscope. Fluorescence in the cytoplasm is indicative of BS203 mRNA. Alternatively, the sections can be visualized by autoradiography.

Example 8: Reverse Transcription PCR

A. One Step RT-PCR Assay. Target-specific primers are designed to detect the above-described target sequences by reverse transcription PCR using methods

known in the art. One step RT-PCR is a sequential procedure that performs both RT and PCR in a single reaction mixture. The procedure is performed in a 200 µl reaction mixture containing 50 mM (N,N,-bis[2-Hydroxyethyl]glycine), pH 8.15, 81.7 mM KOAc, 33.33 mM KOH, 0.01 mg/ml bovine serum albumin, 0.1 mM ethylene diaminetetraacetic acid, 0.02 mg/ml NaN<sub>3</sub>, 8% w/v glycerol, 150 µM each of dNTP, 0.25 µM each primer, 5U rTth polymerase, 3.25 mM Mn(OAc)<sub>2</sub> and 5 µl of target RNA (see Example 3). Since RNA and the rTth polymerase enzyme are unstable in the presence of Mn(OAc)<sub>2</sub>, the Mn(OAc)<sub>2</sub> should be added just before target addition. Optimal conditions for cDNA synthesis and thermal cycling readily can be determined by those skilled in the art. The reaction is incubated in a Perkin-Elmer Thermal Cycler 480. Optimal conditions for cDNA synthesis and thermal cycling can readily be determined by those skilled in the art. Conditions which may be found useful include cDNA synthesis at 60°-70°C for 15-45 min and 30-45 amplification cycles at 94°C, 1 min; 55°-70°C, 1 min; 72°C, 2 min. One step RT-PCR also may be performed by using a dual enzyme procedure with Taq polymerase and a reverse transcriptase enzyme, such as MMLV or AMV RT enzymes.

B. Traditional RT-PCR. Alternatively, a traditional two-step RT-PCR reaction may be performed, as described by K.Q. Hu et al., *Virology* 181:721-726 (1991), as follows. The extracted mRNA is transcribed in a 25 µl reaction mixture containing 10 mM Tris-HCl, pH 8.3, 5 mM MgCl<sub>2</sub>, 500 µM dNTP, 20 U RNasin, 1 µM antisense primer and 25 U AMV (avian myeloblastosis virus) or MMLV (Moloney murine leukemia virus) reverse transcriptase. Reverse transcription is performed at 37-45°C for 30-60 min, followed by further incubation at 95°C for 5 min to inactivate the RT. PCR is performed using 10 µl of the cDNA reaction in a final PCR reaction volume of 50 µl containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl<sub>2</sub>, 200 µM dNTP, 0.5 µM of each primer and 2.5 U of Taq polymerase. Optimal conditions for cDNA synthesis and thermal cycling can be readily determined by those skilled in the art. The reaction is incubated in a Perkin-Elmer Thermal Cycler 480. Conditions which may be found useful include 30-45 cycles of amplification (94°C, 1 min; 55-70°C, 1 min; 72°C, 2 min), final extension (72°C, 10 min) and soak at 4°C.

C. PCR Fragment Analysis. The correct products then can be verified by size determination using gel electrophoresis with fluorescent intercalators or by Southern, dot or slot blot analysis using a labeled probe against the internal sequences of the PCR product. The probes also may be polynucleotides analogs, such as morpholinos or peptide nucleic acids analogs (PNAs). Detection of a

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product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof, is indicative of the presence of BS203 mRNA(s), suggesting a diagnosis of a breast tissue disease or condition, such as breast cancer.

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Example 9: OH-PCR

A. Probe selection and Labeling. Target-specific primers and probes are designed to detect the above-described target sequences by oligonucleotide hybridization PCR. International Publication Nos WO 92/10505, published 25 June 10 1992, and WO 92/11388, published 9 July 1992, teach methods for labeling oligonucleotides at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. For example, see N. T. Thuong et al., Tet. Letters 29(46):5905-5908 (1988); or J. S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL- 15 7-246,688) (1989). Preferably, probes are labeled at their 3' end to prevent participation in PCR and the formation of undesired extension products. For one step OH-PCR the probe should have a  $T_M$  at least 15°C below the  $T_M$  of the primers. The primers and probes are utilized as specific binding members with or without detectable labels using standard phosphoramidite chemistry and/or post-synthetic labeling methods which are well-known to one skilled in the art. 20

B. One Step Oligo Hybridization PCR. OH-PCR is performed on a 200 µl reaction containing 50 mM (N,N,-bis[2-Hydroxyethyl]glycine), pH 8.15, 81.7 mM KOAc, 33.33 mM KOH, 0.01 mg/ml bovine serum albumin, 0.1 mM ethylene 25 diaminetetraacetic acid, 0.02 mg/ml  $\text{NaN}_3$ , 8% w/v glycerol, 150 µM each of dNTP, 0.25 µM each primer, 3.75 nM probe, 5U rTth polymerase, 3.25 mM  $\text{Mn}(\text{OAc})_2$  and 5 µl blood equivalents of target (see Example 3). Since RNA and the rTth polymerase enzyme are unstable in the presence of  $\text{Mn}(\text{OAc})_2$ , the  $\text{Mn}(\text{OAc})_2$  should be added just before target addition. The reaction is incubated in a Perkin-Elmer 30 Thermal Cycler 480. Optimal conditions for cDNA synthesis and thermal cycling can be readily determined by those skilled in the art. Conditions which may be found useful include cDNA synthesis (60°C, 30 min), 30-45 amplification cycles (94°C, 40 sec; 55-70°C, 60 sec), oligo-hybridization (97°C, 5 min; 15°C, 5 min; 15°C soak). The correct reaction product contains at least one of the strands of the 35 PCR product and an internally hybridized probe.

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C. OH-PCR Product Analysis. Amplified reaction products are detected on an LCx<sup>®</sup> analyzer system (available from Abbott Laboratories, Abbott Park, IL). Briefly, the correct reaction product is captured by an antibody labeled microparticle at a capturable site on either the PCR product strand or the hybridization probe, and the complex is detected by binding of a detectable antibody conjugate to either a detectable site on the probe or the PCR strand. Only a complex containing a PCR strand hybridized with the internal probe is detectable. The detection of this complex then is indicative of the presence of BS203 mRNA, suggesting a diagnosis of a breast disease or condition, such as breast cancer.

Many other detection formats exist which can be used and/or modified by those skilled in the art to detect the presence of amplified or non-amplified BS203-derived nucleic acid sequences including, but not limited to, ligase chain reaction (LCR, Abbott Laboratories, Abbott Park, IL); Q-beta replicase (Gene-Trak<sup>™</sup>, Naperville, Illinois), branched chain reaction (Chiron, Emeryville, CA) and strand displacement assays (Becton Dickinson, Research Triangle Park, NC).

Example 10: Synthetic Peptide Production

Synthetic peptides are modeled and then prepared based upon the predicted amino acid sequence of the BS203 polypeptide consensus sequence (see example 1). Peptides modeled for BS203 include SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof derived from SEQUENCE ID NO 17. All peptides are synthesized on an ABI Peptide Synthesizer (available from Applied Biosystems, Foster City, CA), Model 431A or similar instrument, using Fmoc chemistry, standard cycles and DCC-HOBT activation. Cleavage and deprotection conditions are as follows: the resin is added to 20 ml trifluoroacetic acid (TFA), 0.3 ml water, 0.2 ml ethanedithiol, 0.2 ml thioanisole and 100 mg phenol, and stirred at room temperature for 1.5 hours. The resin then is filtered by suction and the peptide is obtained by precipitation of the TFA solution with ether followed by filtration. Each peptide is purified via reverse-phase preparative HPLC using a water/acetonitrile/0.1% TFA gradient and lyophilized. The product is confirmed by mass spectrometry (see Example 12).

Disulfide bond formation is accomplished using auto-oxidation conditions, as follows: the peptide is dissolved in a minimum amount of DMSO (approximately 10 ml) before adding buffer (0.1 M Tris-HCl, pH 6.2) to a concentration of 0.3 - 0.8 mg/ml. The reaction is monitored by HPLC until complete formation of the disulfide bond, followed by reverse-phase preparative HPLC using a

water/acetonitrile/0.1% TFA gradient and lyophilization. The product then is confirmed by mass spectrometry (see Example 12).

The purified peptides can be conjugated to Keyhole Limpet Hemocyanin or other immunoreactive molecule with glutaraldehyde, mixed with adjuvant, and injected into animals.

Example 11a: Expression of Protein in a Cell Line Using Plasmid 577

A. Construction of a BS203 Expression Plasmid. Plasmid 577, described in U.S. patent application Serial No. 08/478,073, filed June 7, 1995 and incorporated herein by reference, has been constructed for the expression of secreted antigens in a permanent cell line. This plasmid contains the following DNA segments: (a) a 2.3 Kb fragment of pBR322 containing bacterial beta-lactamase and origin of DNA replication; (b) a 1.8 Kb cassette directing expression of a neomycin resistance gene under control of HSV-1 thymidine kinase promoter and poly-A addition signals; (c) a 1.9 Kb cassette directing expression of a dihydrofolate reductase gene under the control of an SV-40 promoter and poly-A addition signals; (d) a 3.5 Kb cassette directing expression of a rabbit immunoglobulin heavy chain signal sequence fused to a modified hepatitis C virus (HCV) E2 protein under the control of the Simian Virus 40 T-Ag promoter and transcription enhancer, the hepatitis B virus surface antigen (HBsAg) enhancer I followed by a fragment of Herpes Simplex Virus-1 (HSV-1) genome providing poly-A addition signals; and (e) a residual 0.7 Kb fragment of Simian Virus 40 genome late region of no function in this plasmid. All of the segments of the vector were assembled by standard methods known to those skilled in the art of molecular biology.

Plasmids for the expression of secretable BS203 proteins are constructed by replacing the hepatitis C virus E2 protein coding sequence in plasmid 577 with that of a BS203 polynucleotide sequence selected from the group consisting of SEQUENCE ID NOS 1-14, and fragments or complements thereof, as follows. Digestion of plasmid 577 with XbaI releases the hepatitis C virus E2 gene fragment. The resulting plasmid backbone allows insertion of the BS203 cDNA insert downstream of the rabbit immunoglobulin heavy chain signal sequence which directs the expressed proteins into the secretory pathway of the cell. The BS203 cDNA fragment is generated by PCR using standard procedures. Encoded in the sense PCR primer sequence is an XbaI site, immediately followed by a 12 nucleotide sequence that encodes the amino acid sequence Ser-Asn-Glu-Leu ("SNEL") to promote signal protease processing, efficient secretion and final product stability in



culture fluids. Immediately following this 12 nucleotide sequence the primer contains nucleotides complementary to template sequences encoding amino acids of the BS203 gene. The antisense primer incorporates a sequence encoding the following eight amino acids just before the stop codons: Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQUENCE ID NO 22). Within this sequence is incorporated a recognition site to aid in analysis and purification of the BS203 protein product. A recognition site (termed "FLAG") that is recognized by a commercially available monoclonal antibody designated anti-FLAG M2 (Eastman Kodak, Co., New Haven, CT) can be utilized, as well as other comparable sequences and their corresponding antibodies. For example, PCR is performed using GeneAmp® reagents obtained from Perkin-Elmer-Cetus, as directed by the supplier's instructions. PCR primers are used at a final concentration of 0.5 µM. PCR is performed on the BS203 plasmid template in a 100 µl reaction for 35 cycles (94°C, 30 seconds; 55°C, 30 seconds; 72°C, 90 seconds) followed by an extension cycle of 72°C for 10 min.

B. Transfection of Dihydrofolate Reductase Deficient Chinese Hamster Ovary Cells. The plasmid described supra is transfected into CHO/dhfr- cells (DXB-111, Uriacio, et al., PNAS 77:4451-4466 (1980)). These cells are available from the A.T.C.C., 12301 Parklawn Drive, Rockville, MD 20852, under Accession No. CRL 9096. Transfection is carried out using the cationic liposome-mediated procedure described by P. L. Felgner et al., PNAS 84:7413-7417 (1987). Particularly, CHO/dhfr- cells are cultured in Ham's F-12 media supplemented with 10% fetal calf serum, L-glutamine (1 mM) and freshly seeded into a flask at a density of 5 - 8 x 10<sup>5</sup> cells per flask. The cells are grown to a confluency of between 60 and 80% for transfection. Twenty micrograms (20µg) of plasmid DNA is added to 1.5 ml of Opti-MEM I medium and 100 µl of Lipofectin Reagent (Gibco-BRL; Grand Island, NY) are added to a second 1.5 ml portion of Opti-MEM I media. The two solutions are mixed and incubated at room temperature for 20 min. After the culture medium is removed from the cells, the cells are rinsed 3 times with 5 ml of Opti-MEM I medium. The Opti-MEM I-Lipofection-plasmid DNA solution then is overlaid onto the cells. The cells are incubated for 3 h at 37°C, after which time the Opti-MEM I-Lipofectin-DNA solution is replaced with culture medium for an additional 24 h prior to selection.

C. Selection and Amplification. One day after transfection, cells are passaged 1:3 and incubated with dhfr/G418 selection medium (hereafter, "F-12 minus medium G"). Selection medium is Ham's F-12 with L-glutamine and without hypoxanthine, thymidine and glycine (JRH Biosciences, Lenexa, Kansas) and 300

µg per ml G418 (Gibco-BRL; Grand Island, NY). Media volume-to-surface area ratios of 5 ml per 25 cm<sup>2</sup> are maintained. After approximately two weeks, DHFR/G418 cells are expanded to allow passage and continuous maintenance in F-12 minus medium G.

5           Amplification of each of the transfected BS203 cDNA sequences is achieved by stepwise selection of DHFR<sup>+</sup>, G418<sup>+</sup> cells with methotrexate (reviewed by R. Schimke, Cell 37:705-713 [1984]). Cells are incubated with F-12 minus medium G containing 150 nM methotrexate (MTX) (Sigma, St. Louis, MO) for approximately two weeks until resistant colonies appear. Further gene amplification is achieved by  
10          selection of 150 nM adapted cells with 5 µM MTX.

D. Antigen Production. F-12 minus medium G supplemented with 5 µM MTX is overlaid onto just confluent monolayers for 12 to 24 h at 37°C in 5% CO<sub>2</sub>. The growth medium is removed and the cells are rinsed 3 times with Dulbecco's phosphate buffered saline (PBS) (with calcium and magnesium) (Gibco-BRL; Grand  
15       Island, NY) to remove the remaining media/serum which may be present. Cells then are incubated with VAS custom medium (VAS custom formulation with L-glutamine with HEPES without phenol red, available from JRH Bioscience; Lenexa, KS, product number 52-08678P), for 1 h at 37°C in 5% CO<sub>2</sub>. Cells then are overlaid with VAS for production at 5 ml per T flask. Medium is removed after seven days  
20       of incubation, retained, and then frozen to await purification with harvests 2, 3 and 4. The monolayers are overlaid with VAS for 3 more seven day harvests.

E. Analysis of Breast Tissue Gene BS203 Antigen Expression. Aliquots of VAS supernatants from the cells expressing the BS203 protein construct are analyzed, either by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using  
25       standard methods and reagents known in the art (Laemmli discontinuous gels), or by mass spectrometry.

F. Purification. Purification of the BS203 protein containing the FLAG sequence is performed by immunoaffinity chromatography using an affinity matrix comprising anti-FLAG M2 monoclonal antibody covalently attached to agarose by  
30       hydrazide linkage (Eastman Kodak Co., New Haven, CT). Prior to affinity purification, protein in pooled VAS medium harvests from roller bottles is exchanged into 50 mM Tris-HCl (pH 7.5), 150 mM NaCl buffer using a Sephadex G-25 (Pharmacia Biotech Inc., Uppsala, Sweden) column. Protein in this buffer is applied to the anti-FLAG M2 antibody affinity column. Non-binding protein is  
35       eluted by washing the column with 50 mM Tris-HCl (pH 7.5), 150 mM NaCl buffer. Bound protein is eluted using an excess of FLAG peptide in 50 mM Tris-

HCl (pH 7.5), 150 mM NaCl. The excess FLAG peptide can be removed from the purified BS203 protein by gel electrophoresis or HPLC.

Although plasmid 577 is utilized in this example, it is known to those skilled in the art that other comparable expression systems, such as CMV, can be utilized herein with appropriate modifications in reagent and/or techniques and are within the skill of the ordinary artisan.

Then, the largest cloned insert containing the coding region of the BS203 gene is sub-cloned into either (i) a eukaryotic expression vector which may contain, for example, a cytomegalovirus (CMV) promoter and/or protein fusible sequences which aid in protein expression and detection, or (ii) a bacterial expression vector containing a superoxide-dismutase (SOD) and CMP-KDO synthetase (CKS) or other protein fusion gene for expression of the protein sequence. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in EPO 0196056, published October 1, 1986, which is incorporated herein by reference and those containing fusion sequences of CKS are described in EPO Publication No. 0331961, published September 13, 1989, which publication is also incorporated herein by reference. This so-purified protein can be used in a variety of techniques, including but not limited to animal immunization studies, solid phase immunoassays, etc.

#### Example 11b: Expression of Protein in a Cell

##### Line using Plasmid pcDNA3.1/Myc-His

A. Construction of a BS203 Expression Plasmid. Plasmid pcDNA3.1/Myc-His (Cat.# V855-20, Invitrogen, Carlsbad, CA) has been constructed, in the past, for the expression of secreted antigens by most mammalian cell lines. Expressed protein inserts are fused to a myc-his peptide tag. The myc-his tag is a 21 residue amino acid sequence having the following sequence: Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-Asn-Met-His-Thr-Glu-His-His-His-His-His-His (SEQUENCE ID NO 23) and comprises a myc epitope and a polyhistidine sequence which are useful for the purification of an expressed fusion protein using either anti-myc or anti-his affinity columns, or metalloprotein binding columns.

Plasmids for the expression of secretable BS203 proteins are constructed by inserting a BS203 polynucleotide sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, SEQUENCE ID NO 6, SEQUENCE ID NO 7, SEQUENCE ID NO 8, SEQUENCE ID NO 9, SEQUENCE ID NO 10,

SEQUENCE ID NO 11, SEQUENCE ID NO 12, SEQUENCE ID NO 13, SEQUENCE ID NO 14, and fragments or complements thereof. Prior to construction of a BS203 expression plasmid, the BS203 cDNA sequence is first cloned into a pCR®-Blunt vector as follows.

5           The BS203 cDNA fragment is generated by PCR using standard procedures. For example, PCR is performed using Stratagene® reagents obtained from Stratagene, La Jolla, CA, as directed by the supplier's instructions. PCR primers are used at a final concentration of 0.5 µM. PCR using 5 U of pfu polymerase (Stratagene) is performed on the BS203 plasmid template (see Example 2) in a 50 µl  
10    reaction for 30 cycles (94°C, 1 min; 65°C, 1.5 min; 72°C, 3 min) followed by an extension cycle at 72°C for 8 min. The sense PCR primer sequence comprises nucleotides which are either complementary to the pINCY vector directly upstream of the BS203 gene insert or which incorporate a 5' EcoRI restriction site, an adjacent downstream protein translation consensus initiator, and a 3' nucleic acid sequence  
15    which is the same sense as the 5'-most end of the BS203 cDNA insert. The antisense primer incorporates a 5' NotI restriction sequence and a sequence complementary to the 3' end of the BS203 cDNA insert just upstream of the 3'-most, in-frame stop codon. Five microliters (5 µl) of the resulting blunt-ended PCR product are ligated into 25 ng of linearized pCR®-Blunt vector (Invitrogen, Carlsbad,  
20    CA) interrupting the lethal ccdB gene of the vector. The resulting ligated vector is transfected into TOP10 *E. coli* (Invitrogen, Carlsbad, CA) using a One Shot™ transformation kit (Invitrogen, Carlsbad, CA) following the supplier's directions. The transfected cells are grown on LB-Kan (50 µg/ml kanamycin) selection plates at 37°C. Only cells containing a plasmid with an interrupted ccdB gene will grow after  
25    transfection (Grant, S.G.N., PNAS USA 87:4645-4649 (1990)). Transfected colonies are picked and grown up in 3 ml of LB-Kan broth at 37°C. Plasmid DNA is isolated using a QIAprep® (Qiagen Inc., Santa Clarita, CA) procedure, as directed by the supplier's instructions. The DNA is cut with EcoRI or SnaBI, and NotI  
30    restriction enzymes to release the BS203 insert fragment. The fragment is run on 1% Seakem® LE agarose/0.5 µg/ml ethidium bromide/TE gel, visualized by UV irradiation, excised and purified using QIAquick™ (Qiagen Inc., Santa Clarita, CA) procedures, as directed by the supplier's instructions.

          The pcDNA3.1/Myc-His plasmid DNA is linearized by digestion with EcoRI or SnaBI, and NotI in the polylinker region of the plasmid DNA. The resulting  
35    plasmid DNA backbone allows insertion of the BS203 purified cDNA fragment, supra, downstream of a CMV promoter which directs expression of the proteins in

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harvested by washing twice with 10 ml cold Dulbecco's PBS and lysing by addition of 1.5 ml of CAT lysis buffer (Boehringer Mannheim, Indianapolis, IN), followed by incubation for 30 min at room temperature. Lysate is transferred to 1.7 ml polypropylene microfuge tubes and centrifuged at 1000 x g for 10 min. The  
5 supernatant is transferred to new cryotubes and stored on ice. Aliquots of cell supernatants and the lysate of the cells expressing the BS203 protein construct are analyzed for the presence of BS203 recombinant protein. The aliquots can be analyzed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), using standard methods and reagents known in the art. See, e.g., J. Sambrook et al.,  
10 supra. The gels can then be blotted onto a solid medium such as nitrocellulose, nytran, or the like, and the BS203 protein band can be visualized using western blotting techniques with anti-myc epitope or anti-histidine monoclonal antibodies (Invitrogen, Carlsbad, CA) or BS203 polyclonal serum (see Example 14). Alternatively, the expressed BS203 recombinant protein can be analyzed by mass  
15 spectrometry (see Example 12).

D. Purification. Purification of the BS203 recombinant protein containing the myc-his sequence is performed using the Xpress® affinity chromatography system (Invitrogen, Carlsbad, CA) containing a nickel-charged agarose resin which specifically binds polyhistidine residues. Supernatants from 10 x 100 mm plates,  
20 prepared as described supra, are pooled and passed over the nickel-charged column. Non-binding protein is eluted by washing the column with 50 mM Tris-HCl (pH 7.5)/150 mM NaCl buffer, leaving only the myc-his fusion proteins. Bound BS203 recombinant protein then is eluted from the column using either an excess of imidazole or histidine, or a low pH buffer. Alternatively, the recombinant protein  
25 can also be purified by binding at the myc-his sequence to an affinity column consisting of either anti-myc or anti-histidine monoclonal antibodies conjugated through a hydrazide or other linkage to an agarose resin and eluting with an excess of myc peptide or histidine, respectively.

The purified recombinant protein can then be covalently cross-linked to a  
30 solid phase, such as N-hydroxysuccinimide-activated sepharose columns (Pharmacia Biotech, Piscataway, NJ), as directed by supplier's instructions. These columns containing covalently linked BS203 recombinant protein, can then be used to purify anti-BS203 antibodies from rabbit or mouse sera (see Examples 13 and 14).

E. Coating Microtiter Plates with BS203 Expressed Proteins. Supernatant  
35 from a 100 mm plate, as described supra, is diluted in an appropriate volume of

PBS. 100 µl of the resulting mixture is placed into each well of a Reacti-Bind™ metal chelate microtiter plate (Pierce, Rockford, IL), incubated at room temperature while shaking, and followed by three washes with 200 µl each of PBS with 0.05% Tween® 20. The prepared microtiter plate can then be used to screen polyclonal antisera for the presence of BS203 antibodies (see Example 17).

Although pcDNA3.1/Myc-His is utilized in this example, it is known to those skilled in the art that other comparable expression systems can be utilized herein with appropriate modifications in reagent and/or techniques and are within the skill of one of ordinary skill in the art. The largest cloned insert containing the coding region of the BS203 gene is sub-cloned into either (i) a eukaryotic expression vector which may contain, for example, a cytomegalovirus (CMV) promoter and/or protein fusible sequences which aid in protein expression and detection, or (ii) a bacterial expression vector containing a superoxide-dismutase (SOD) and CMP-KDO synthetase (CKS) or other protein fusion gene for expression of the protein sequence. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in European patent application No. EP 0 196 056, published October 1, 1986, which is incorporated herein by reference, and vectors containing fusion sequences of CKS are described in European patent application No. EP 0 331 961, published September 13, 1989, which publication is also incorporated herein by reference. The purified protein can be used in a variety of techniques, including but not limited to, animal immunization studies, solid phase immunoassays, etc.

#### Example 12: Chemical Analysis of Breast Tissue Proteins

A. Analysis of Tryptic Peptide Fragments Using MS. Sera from patients with breast disease such as breast cancer, sera from patients with no breast disease, extracts of breast tissues or cells from patients with breast disease such as breast cancer, extracts of breast tissues or cells from patients with no breast disease, and extracts of tissues or cells from other non-diseased or diseased organs of patients, are run on a polyacrylamide gel using standard procedures and stained with Coomassie Blue. Sections of the gel suspected of containing the unknown polypeptide are excised and subjected to an in-gel reduction, acetamidation and tryptic digestion. P. Jenö et al, Anal. Bio. 224:451-455 (1995) and J. Rosenfeld et al, Anal. Bio. 203:173-179 (1992). The gel sections are washed with 100 mM NH<sub>4</sub>HCO<sub>3</sub> and acetonitrile. The shrunken gel pieces are swollen in digestion buffer (50 mM NH<sub>4</sub>HCO<sub>3</sub>, 5 mM CaCl<sub>2</sub> and 12.5 µg/ml trypsin) at 4°C for 45 min. The

supernatant is aspirated and replaced with 5 to 10 µl of digestion buffer without trypsin and allowed to incubate overnight at 37°C. Peptides are extracted with 3 changes of 5% formic acid and acetonitrile and evaporated to dryness. The peptides are adsorbed to approximately 0.1 µl of POROS R2 sorbent (Perseptive Biosystems, Framingham, Massachusetts) trapped in the tip of a drawn gas chromatography capillary tube by dissolving them in 10 µl of 5% formic acid and passing it through the capillary. The adsorbed peptides are washed with water and eluted with 5% formic acid in 60% methanol. The eluant is passed directly into the spraying capillary of an API III mass spectrometer (Perkin-Elmer Sciex, Thornhill, Ontario, Canada) for analysis by nano-electrospray mass spectrometry. M. Wilm et al., Int. J. Mass Spectrom. Ion Process 136:167-180 (1994) and M. Wilm et al., Anal. Chem. 66:1-8 (1994). The masses of the tryptic peptides are determined from the mass spectrum obtained off the first quadrupole. Masses corresponding to predicted peptides can be further analyzed in MS/MS mode to give the amino acid sequence of the peptide.

B. Peptide Fragment Analysis Using LC/MS. The presence of polypeptides predicted from mRNA sequences found in hyperplastic disease tissues also can be confirmed using liquid chromatography/tandem mass spectrometry (LC/MS/MS). D. Hess et al., METHODS, A Companion to Methods in Enzymology 6:227-238 (1994). The serum specimen or tumor extract from the patient is denatured with SDS and reduced with dithiothreitol (1.5 mg/ml) for 30 min at 90°C followed by alkylation with iodoacetamide (4 mg/ml) for 15 min at 25°C. Following acrylamide electrophoresis, the polypeptides are electroblotted to a cationic membrane and stained with Coomassie Blue. Following staining, the membranes are washed and sections thought to contain the unknown polypeptides are cut out and dissected into small pieces. The membranes are placed in 500 µl microcentrifuge tubes and immersed in 10 to 20 µl of proteolytic digestion buffer (100 mM Tris-HCl, pH 8.2, containing 0.1 M NaCl, 10% acetonitrile, 2 mM CaCl<sub>2</sub> and 5 µg/ml trypsin) (Sigma, St. Louis, MO). After 15 h at 37°C, 3 µl of saturated urea and 1 µl of 100 µg/ml trypsin are added and incubated for an additional 5 h at 37°C. The digestion mixture is acidified with 3 µl of 10% trifluoroacetic acid and centrifuged to separate supernatant from membrane. The supernatant is injected directly onto a microbore, reverse phase HPLC column and eluted with a linear gradient of acetonitrile in 0.05% trifluoroacetic acid. The eluate is fed directly into an electrospray mass spectrometer, after passing through a stream splitter if necessary to adjust the volume

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of material. The data is analyzed following the procedures set forth in Example 12, Section A.

Example 13: Gene Immunization Protocol

5           A. In Vivo Antigen Expression. Gene immunization circumvents protein purification steps by directly expressing an antigen in vivo after inoculation of the appropriate expression vector. Also, production of antigen by this method may allow correct protein folding and glycosylation since the protein is produced in mammalian tissue. The method utilizes insertion of the gene sequence into a plasmid  
10 which contains a CMV promoter, expansion and purification of the plasmid and injection of the plasmid DNA into the muscle tissue of an animal. Preferred animals include mice and rabbits. See, for example, H. Davis et al., Human Molecular Genetics 2:1847-1851 (1993). After one or two booster immunizations, the animal can then be bled, ascites fluid collected, or the animal's spleen can be harvested for  
15 production of hybridomas.

B. Plasmid Preparation and Purification. BS203 cDNA sequences are generated from the BS203 cDNA-containing vector using appropriate PCR primers containing suitable 5' restriction sites following the procedures described in Example 11. The PCR product is cut with appropriate restriction enzymes and inserted into a  
20 vector which contains the CMV promoter (for example, pRc/CMV or pcDNA3 vectors from Invitrogen, San Diego, CA). This plasmid then is expanded in the appropriate bacterial strain and purified from the cell lysate using a CsCl gradient or a Qiagen plasmid DNA purification column. All these techniques are familiar to one of ordinary skill in the art of molecular biology.

25           C. Immunization Protocol. Anesthetized animals are immunized intramuscularly with 0.1-100 µg of the purified plasmid diluted in PBS or other DNA uptake enhancers (Cardiotoxin, 25% sucrose). See, for example, H. Davis et al, Human Gene Therapy 4:733-740 (1993); and P. W. Wolff et al, Biotechniques 11:474-485 (1991). One to two booster injections are given at monthly intervals.

30           D. Testing and Use of Antiserum. Animals are bled and the resultant sera tested for antibody using peptides synthesized from the known gene sequence (see Example 16) using techniques known in the art, such as western blotting or EIA techniques. Antisera produced by this method can then be used to detect the presence of the antigen in a patient's tissue or cell extract, or in a patient's serum, by  
35 ELISA or Western blotting techniques, such as those described in Examples 15 through 18.

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Example 14: Production of Antibodies Against BS203

5        A. Production of Polyclonal Antisera. Antiserum against BS203 is prepared  
by injecting appropriate animals with peptides whose sequences are derived from  
that of the predicted amino acid sequence of the BS203 consensus sequence  
(SEQUENCE ID NO 14). The synthesis of peptides (SEQUENCE ID NO 18,  
SEQUENCE ID NO 19, SEQUENCE ID NO 20, and SEQUENCE ID NO 21) is  
10 described in Example 10. Peptides used as immunogen either can be conjugated to a  
carrier such as keyhole limpet hemocyanine (KLH), prepared as described  
hereinbelow, or unconjugated (i.e., not conjugated to a carrier such as KLH).

1. Peptide Conjugation. Peptide is conjugated to maleimide activated  
keyhole limpet hemocyanine (KLH, commercially available as Imject®, available  
15 from Pierce Chemical Company, Rockford, IL). Imject® contains about 250 moles  
of reactive maleimide groups per mole of hemocyanine. The activated KLH is  
dissolved in phosphate buffered saline (PBS, pH 8.4) at a concentration of about 7.7  
mg/ml. The peptide is conjugated through cysteines occurring in the peptide  
sequence, or to a cysteine previously added to the synthesized peptide in order to  
20 provide a point of attachment. The peptide is dissolved in dimethyl sulfoxide  
(DMSO, Sigma Chemical Company, St. Louis, MO) and reacted with the activated  
KLH at a mole ratio of about 1.5 moles of peptide per mole of reactive maleimide  
attached to the KLH. A procedure for the conjugation of peptide (SEQUENCE ID  
NO 18) is provided hereinbelow. It is known to the ordinary artisan that the  
25 amounts, times and conditions of such a procedure can be varied to optimize peptide  
conjugation.

The conjugation reaction described hereinbelow is based on obtaining 3 mg  
of KLH peptide conjugate ("conjugated peptide"), which contains about 0.77  
µmoles of reactive maleimide groups. This quantity of peptide conjugate usually is  
30 adequate for one primary injection and four booster injections for production of  
polyclonal antisera in a rabbit. Briefly, peptide (SEQUENCE ID NO 18) is  
dissolved in DMSO at a concentration of 1.16 µmoles/100 µl of DMSO. One  
hundred microliters (100 µl) of the DMSO solution is added to 380 µl of the  
activated KLH solution prepared as described hereinabove, and 20 µl of PBS (pH  
35 8.4) is added to bring the volume to 500 µl. The reaction is incubated overnight at  
room temperature with stirring. The extent of reaction is determined by measuring

the amount of unreacted thiol in the reaction mixture. The difference between the starting concentration of thiol and the final concentration is assumed to be the concentration of peptide which has coupled to the activated KLH. The amount of remaining thiol is measured using Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid), Pierce Chemical Company, Rockford, IL). Cysteine standards are made at a concentration of 0, 0.1, 0.5, 2, 5 and 20 mM by dissolving 35 mg of cysteine HCl (Pierce Chemical Company, Rockford, IL) in 10 ml of PBS (pH 7.2) and diluting the stock solution to the desired concentration(s). The photometric determination of the concentration of thiol is accomplished by placing 200 µl of PBS (pH 8.4) in each well of an Immulon 2® microwell plate (Dynex Technologies, Chantilly, VA). Next, 10 µl of standard or reaction mixture is added to each well. Finally, 20 µl of Ellman's reagent at a concentration of 1 mg/ml in PBS (pH 8.4) is added to each well. The wells are incubated for 10 minutes at room temperature, and the absorbance of all wells is read at 415 nm with a microplate reader (such as the BioRad Model 3550, BioRad, Richmond, CA). The absorbance of the standards is used to construct a standard curve and the thiol concentration of the reaction mixture is determined from the standard curve. A decrease in the concentration of free thiol is indicative of a successful conjugation reaction. Unreacted peptide is removed by dialysis against PBS (pH 7.2) at room temperature for 6 hours. The conjugate is stored at 2-8°C if it is to be used immediately; otherwise, it is stored at -20°C or colder.

2. Animal Immunization. Female white New Zealand rabbits weighing 2 kg or more are used for raising polyclonal antiserum. Generally, one animal is immunized per unconjugated or conjugated peptide (prepared as described hereinabove). One week prior to the first immunization, 5 to 10 ml of blood is obtained from the animal to serve as a non-immune prebleed sample.

Unconjugated or conjugated peptide is used to prepare the primary immunogen by emulsifying 0.5 ml of the peptide at a concentration of 2 mg/ml in PBS (pH 7.2) which contains 0.5 ml of complete Freund's adjuvant (CFA) (Difco, Detroit, MI). The immunogen is injected into several sites of the animal via subcutaneous, intraperitoneal, and/or intramuscular routes of administration. Four weeks following the primary immunization, a booster immunization is administered. The immunogen used for the booster immunization dose is prepared by emulsifying 0.5 ml of the same unconjugated or conjugated peptide used for the primary immunogen, except that the peptide now is diluted to 1 mg/ml with 0.5 ml of incomplete Freund's adjuvant (IFA) (Difco, Detroit, MI). Again, the booster dose is

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administered into several sites and can utilize subcutaneous, intraperitoneal and intramuscular types of injections. The animal is bled (5 ml) two weeks after the booster immunization and the serum is tested for immunoreactivity to the peptide, as described below. The booster and bleed schedule is repeated at 4 week intervals  
5 until an adequate titer is obtained. The titer or concentration of antiserum is determined by microtiter EIA as described in Example 17, below. An antibody titer of 1:500 or greater is considered an adequate titer for further use and study.

B. Production of Monoclonal Antibody.

1. Immunization Protocol. Mice are immunized using immunogens  
10 prepared as described hereinabove, except that the amount of the unconjugated or conjugated peptide for monoclonal antibody production in mice is one-tenth the amount used to produce polyclonal antisera in rabbits. Thus, the primary immunogen consists of 100 µg of unconjugated or conjugated peptide in 0.1 ml of CFA emulsion; while the immunogen used for booster immunizations consists of 50  
15 µg of unconjugated or conjugated peptide in 0.1 ml of IFA. Hybridomas for the generation of monoclonal antibodies are prepared and screened using standard techniques. The methods used for monoclonal antibody development follow procedures known in the art such as those detailed in Kohler and Milstein, Nature 256:494 (1975) and reviewed in J.G.R. Hurrel, ed., Monoclonal Hybridoma  
20 Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL (1982). Another method of monoclonal antibody development which is based on the Kohler and Milstein method is that of L.T. Mimms et al., Virology 176:604-619 (1990), which is incorporated herein by reference.

The immunization regimen (per mouse) consists of a primary immunization  
25 with additional booster immunizations. The primary immunogen used for the primary immunization consists of 100 µg of unconjugated or conjugated peptide in 50 µl of PBS (pH 7.2) previously emulsified in 50 µl of CFA. Booster immunizations performed at approximately two weeks and four weeks post primary immunization consist of 50 µg of unconjugated or conjugated peptide in 50 µl of  
30 PBS (pH 7.2) emulsified with 50 µl IFA. A total of 100 µl of this immunogen is inoculated intraperitoneally and subcutaneously into each mouse. Individual mice are screened for immune response by microtiter plate enzyme immunoassay (EIA) as described in Example 17 approximately four weeks after the third immunization. Mice are inoculated either intravenously, intrasplenically or intraperitoneally with 50  
35 µg of unconjugated or conjugated peptide in PBS (pH 7.2) approximately fifteen weeks after the third immunization..

Three days after this intravenous boost, splenocytes are fused with, for example, Sp2/0-Ag14 myeloma cells (Milstein Laboratories, England) using the polyethylene glycol (PEG) method. The fusions are cultured in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% fetal calf serum (FCS), plus 1% hypoxanthine, aminopterin and thymidine (HAT). Bulk cultures are screened by microtiter plate EIA following the protocol in Example 17. Clones reactive with the peptide used as an immunogen and non-reactive with other peptides (i.e., peptides of BS203 not used as the immunogen) are selected for final expansion. Clones thus selected are expanded, aliquoted and frozen in IMDM containing 10% FCS and 10% dimethyl-sulfoxide.

2. Production of Ascites Fluid Containing Monoclonal Antibodies. Frozen hybridoma cells prepared as described hereinabove are thawed and placed into expansion culture. Viable hybridoma cells are inoculated intraperitoneally into Pristane treated mice. Ascitic fluid is removed from the mice, pooled, filtered through a 0.2  $\mu$  filter and subjected to an immunoglobulin class G (IgG) analysis to determine the volume of the Protein A column required for the purification.

3. Purification of Monoclonal Antibodies From Ascites Fluid. Briefly, filtered and thawed ascites fluid is mixed with an equal volume of Protein A sepharose binding buffer (1.5 M glycine, 3.0 M NaCl, pH 8.9) and refiltered through a 0.2  $\mu$  filter. The volume of the Protein A column is determined by the quantity of IgG present in the ascites fluid. The eluate then is dialyzed against PBS (pH 7.2) overnight at 2-8°C. The dialyzed monoclonal antibody is sterile filtered and dispensed in aliquots. The immunoreactivity of the purified monoclonal antibody is confirmed by determining its ability to specifically bind to the peptide used as the immunogen by use of the EIA microtiter plate assay procedure of Example 17. The specificity of the purified monoclonal antibody is confirmed by determining its lack of binding to irrelevant peptides such as peptides of BS203 not used as the immunogen. The purified anti-BS203 monoclonal thus prepared and characterized is placed at either 2-8°C for short term storage or at -80°C for long term storage.

4. Further Characterization of Monoclonal Antibody. The isotype and subtype of the monoclonal antibody produced as described hereinabove can be determined using commercially available kits (available from Amersham, Inc., Arlington Heights, IL). Stability testing also can be performed on the monoclonal antibody by placing an aliquot of the monoclonal antibody in continuous storage at 2-8°C and assaying optical density (OD) readings throughout the course of a given period of time.

	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2
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Alternatively, spleens can be harvested and used in the production of hybridomas to produce monoclonal antibodies following routine methods known in the art as described hereinabove.

35 Tissue samples are homogenized in SDS-PAGE sample buffer (50 mM Tris-HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10%

25

The immunoreactivity of antiserum preferably obtained from rabbits or mice as described in Example 13 or Example 14 is determined by means of a microtiter plate EIA, as follows. Synthetic peptides prepared as described in Example 10 or recombinant proteins prepared as described in Example 11 are dissolved in carbonate buffer (50mM, pH 9.6) to a final concentration of 2 µg/ml. Next, 100 µl of the peptide or protein solution is placed in each well of an Immulon 2® microtiter plate (Dynex Technologies, Chantilly, VA). The plate is incubated overnight at room temperature and then washed four times with deionized water. The wells are blocked by adding 125 µl of a suitable protein blocking agent, such as 3% Superblock® (Pierce Chemical Company, Rockford, IL), in phosphate buffered

saline (PBS, pH 7.4) to each well and then immediately discarding the solution. This blocking procedure is performed three times. Antiserum obtained from immunized rabbits or mice prepared as previously described is diluted in a protein blocking agent (e.g., a 3% Superblock<sup>®</sup> solution) in PBS containing 0.05% Tween-20<sup>®</sup> (monolaurate polyoxyethylene ether) (Sigma Chemical Company, St. Louis, MO) and 0.05% sodium azide at dilutions of 1:500, 1:2500, 1:12,500, 1:62,500 and 1:312,500 and placed in each well of the coated microtiter plate. The wells then are incubated for three hours at room temperature. Each well is washed four times with deionized water. One hundred  $\mu$ l of alkaline phosphatase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG antiserum (Southern Biotech, Birmingham, AB), diluted 1:20,000 in 3% Superblock<sup>®</sup> solution in phosphate buffered saline containing 0.05% Tween 20<sup>®</sup> and 0.05% sodium azide, is added to each well. The wells are incubated for two hours at room temperature. Next, each well is washed four times with deionized water. One hundred microliters (100  $\mu$ l) of paranitrophenyl phosphate substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) then is added to each well. The wells are incubated for thirty minutes at room temperature. The absorbance at 405 nm is read of each well. Positive reactions are identified by an increase in absorbance at 405 nm in the test well above that absorbance given by a non-immune serum (negative control). A positive reaction is indicative of the presence of detectable anti-BS203 antibodies.

#### Example 18: Coating of Solid Phase Particles

A. Coating of Microparticles with Antibodies Which Specifically Bind to BS203 Antigen. Affinity purified antibodies which specifically bind to BS203 protein (see Example 15) are coated onto microparticles of polystyrene, carboxylated polystyrene, polymethylacrylate or similar particles having a radius in the range of about 0.1 to 20  $\mu$ m. Microparticles may be either passively or actively coated. One coating method comprises coating EDAC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Aldrich Chemical Co., Milwaukee, WI) activated carboxylated latex microparticles with antibodies which specifically bind to BS203 protein, as follows. Briefly, a final 0.375% solid suspension of resin washed carboxylated latex microparticles (available from Bangs Laboratories, Carmel, IN or Serodyn, Indianapolis, IN) are mixed in a solution containing 50 mM MES buffer, pH 4.0 and 150 mg/l of affinity purified anti-BS203 antibody (see Example 14) for 15 min in an appropriate container. EDAC coupling agent is added to a final concentration of 5.5  $\mu$ g/ml to the mixture and mixed for 2.5 h at room temperature.



The microparticles then are washed with 8 volumes of a Tween 20®/sodium phosphate wash buffer (pH 7.2) by tangential flow filtration using a 0.2 µm Microgon Filtration module. Washed microparticles are stored in an appropriate buffer which usually contains a dilute surfactant and irrelevant protein as a blocking agent, until needed.

B. Coating of 1/4 Inch Beads. Antibodies which specifically bind to BS203-antigen also may be coated on the surface of 1/4 inch polystyrene beads by routine methods known in the art (Snitman et al, US Patent 5,273,882, incorporated herein by reference) and used in competitive binding or EIA sandwich assays.

Polystyrene beads first are cleaned by ultrasonicing them for about 15 seconds in 10 mM NaHCO<sub>3</sub> buffer at pH 8.0. The beads then are washed in deionized water until all fines are removed. Beads then are immersed in an antibody solution in 10 mM carbonate buffer, pH 8 to 9.5. The antibody solution can be as dilute as 1 µg/ml in the case of high affinity monoclonal antibodies or as concentrated as about 500 µg/ml for polyclonal antibodies which have not been affinity purified. Beads are coated for at least 12 hours at room temperature, and then they are washed with deionized water. Beads may be air dried or stored wet (in PBS, pH 7.4). They also may be overcoated with protein stabilizers (such as sucrose) or protein blocking agents used as non-specific binding blockers (such as irrelevant proteins, Carnation skim milk, Superblock®, or the like).

Example 19: Microparticle Enzyme Immunoassay (MEIA)

BS203 antigens are detected in patient test samples by performing a standard antigen competition EIA or antibody sandwich EIA and utilizing a solid phase such as microparticles (MEIA). The assay can be performed on an automated analyzer such as the IMx® Analyzer (Abbott Laboratories, Abbott Park, IL).

A. Antibody Sandwich EIA. Briefly, samples suspected of containing BS203 antigen are incubated in the presence of anti-BS203 antibody-coated microparticles (prepared as described in Example 17) in order to form antigen/antibody complexes. The microparticles then are washed and an indicator reagent comprising an antibody conjugated to a signal generating compound (i.e., enzymes such as alkaline phosphatase or horseradish peroxidase) is added to the antigen/antibody complexes or the microparticles and incubated. The microparticles are washed and the bound antibody/antigen/antibody complexes are detected by adding a substrate (e.g., 4-methyl umbelliferyl phosphate (MUP), or OPD/peroxide, respectively), that reacts with the signal generating compound to generate a

measurable signal. An elevated signal in the test sample, compared to the signal generated by a negative control, detects the presence of BS203 antigen. The presence of BS203 antigen in the test sample is indicative of a diagnosis of a breast disease or condition, such as breast cancer.

- 5            B. Competitive Binding Assay. The competitive binding assay uses a peptide or protein that generates a measurable signal when the labeled peptide is contacted with an anti-peptide antibody coated microparticle. This assay can be performed on the IMx<sup>®</sup> Analyzer (available from Abbott Laboratories, Abbott Park, IL). The labeled peptide is added to the BS203 antibody-coated microparticles
- 10 (prepared as described in Example 17) in the presence of a test sample suspected of containing BS203 antigen, and incubated for a time and under conditions sufficient to form labeled BS203 peptide (or labeled protein) / bound antibody complexes and/or patient BS203 antigen / bound antibody complexes. The BS203 antigen in the test sample competes with the labeled BS203 peptide (or BS203 protein) for
- 15 binding sites on the microparticle. BS203 antigen in the test sample results in a lowered binding of labeled peptide and antibody coated microparticles in the assay since antigen in the test sample and the BS203 peptide or BS203 protein compete for antibody binding sites. A lowered signal (compared to a control) indicates the presence of BS203 antigen in the test sample. The presence of BS203 antigen
- 20 suggests the diagnosis of a breast disease or condition, such as breast cancer.

- The BS203 polynucleotides and the proteins encoded thereby which are provided and discussed hereinabove are useful as markers of breast tissue disease, especially breast cancer. Tests based upon the appearance of this marker in a test
- 25 sample such as blood, plasma or serum can provide low cost, non-invasive, diagnostic information to aid the physician to make a diagnosis of cancer, to help select a therapy protocol, or to monitor the success of a chosen therapy. This marker may appear in readily accessible body fluids such as blood, urine or stool as antigens derived from the diseased tissue which are detectable by immunological methods.
- 30 This marker may be elevated in a disease state, altered in a disease state, or be a normal protein of the breast which appears in an inappropriate body compartment.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: Russell, John  
Colpitts, Tracey
- (ii) TITLE OF THE INVENTION: REAGENTS AND METHODS USEFUL  
FOR DETECTING DISEASE OF THE BREAST
- (iii) NUMBER OF SEQUENCES: 23
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Abbott Laboratories  
(B) STREET: 100 Abbott Park Road  
(C) CITY: Abbott Park  
(D) STATE: IL  
(E) COUNTRY: USA  
(F) ZIP: 60064-3500
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Diskette  
(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: DOS  
(D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Becker, Cheryl L  
(B) REGISTRATION NUMBER: 35,441  
(C) REFERENCE/DOCKET NUMBER: 6131.US.01
- (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 847/935-1729  
(B) TELEFAX: 847/938-2623  
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 262 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGAAGANCTG	CTTCGAGGAG	GAGCTCATCT	GCNCTATCTG	CCTGCACGTT	TTCGTGGAGC	60
CAGTGCAGCT	GCCGTGCAAA	CACANCTTCT	GCCGGGGCTG	CATCGGCGAG	GCGTGGGCCA	120
AGGACAGCGG	CCTCGTACGC	TGCCAGAGT	GCAACCAGGC	CTACAACCAG	ANGCCGGGCC	180
TGGAGAAGAA	CCTGAAGCTC	ACCAACATCG	TGGAGAAGTT	CAATGCCCTG	CNCGTGGAGA	240
NGCCGCCGGC	GGCGCTGCAC	TG				262

(2) INFORMATION FOR SEQ ID NO:2:

039083 03459  
66920 6805260

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 263 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTTTTCGTGG	AGCCAGTGCA	GCTGCCGTGC	AAACACAAC	TCTGCCGGGG	CTGCATCGGC	60
GAGGCGTGG	CCAAGGACAG	CGGCCTCGTA	CGCTGCCCAG	AGTGCAACCA	GGCCNACAAC	120
CAGAAGCCGG	GCCTGGNGAA	GAACCTGAAG	CTCACCACCA	TCGTGGAGAA	GTTCAATGCC	180
CTGCACGTGG	AGAANCCGCC	GGCGGCGCTG	CACTGCGTGT	TCTGCCGCCG	CGCCCCCCC	240
CTGCCCCGCG	AGAAGGTCTG	CCT				263

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 254 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATCGGCGAGG	CGTGGGCCAA	GGACAGCNGC	CTCGTACGCT	GCCCAGAGTG	CAACCAGGCC	60
TACAACCAGA	AGCCGGGCCT	GGAGAAGAAC	CTGAAGCTCA	CCAACATCGT	GGAGAAGTTC	120
AATGCCCTGC	ACGTGGAGAN	GCCGCCGCN	GCGCTGCACT	GCGTGTTCTG	CCGCCCGCGG	180
CCCCGCTGC	CCGCGCAGAA	GGTCTGCCTG	CGCTGCGAGG	CGCCCTGCTG	CCAGTCCCAC	240
GTGCAGACGC	ACCT					254

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 273 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGCGCTGCGA	GGCGCCCTGC	TGCCAGTCCC	ACGTGCAGAC	GCACCTGCAG	CAGCCCTCCA	60
CCGCCCCGCG	GCACCTCCTG	GTGGAGGCGG	ACGACGTGCG	GGCCTGGAGC	TGCCCCGAGC	120
ACAACGCCTA	CCGCCTCTAC	CACTGCGAGG	CCGAGCAGGT	GGCCGTGTGC	CAGTACTGCT	180
GCTACTACAG	CGGCGCGCAT	CAGGGACACT	CGGTGTGCGA	CGTGGAGATC	CGAAGGAATG	240
AAATCCGGAA	GATGCTCATG	AAGCAGCAGG	ACC			273

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 240 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGTGTGCGAC	GTGGAGATCC	GAAGGAATGA	AATCCGGAAG	ATGCTCATGA	AGCAGCAGGA	60
CCGGCTGGAG	GAGCGAGAGC	AGGACATTGA	GGACCAGCTG	TACAAACTCG	AGTCAGACAA	120
GCGCCTGGTG	GAGGAGAAAG	TGAACCAACT	GAAGGAGGAA	GTTCCGGCTG	AGTACGAGAA	180
GCTGCACCAG	CTGCTGGACG	AGGACCTGCG	GCAGACAGTG	GAGGTCTTAG	ACAAGGCCCA	240

09260883 094699

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 268 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTGGAGGAGA	AAGTGAACCA	ACTGAAGGAG	GAAGTTCGGC	TGCAGTACGA	GAAGCTGCAC	60
CAGCTGCTGG	ACGAGGACCT	GCGGCAGACA	GTGGAGGTCC	TAGACAAGGC	CCAGGCCAAG	120
TTCTGCAGCG	AGAACGCAGC	GCAGGCGCTG	CACCTCGGGG	AGCGCATGCA	GGAGGCCAAG	180
AAGCTGCTGG	GCTCCCTGCA	GCTGCTCTTT	GATAAGACGG	AGGATGTCAG	CTTCATGAAG	240
AACACCAAGT	CTGTGAAAAT	CCTGATGG				268

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 239 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGAGAACGCA	GCGCAGGCGC	TGCACCTCGG	GGAGCGCATG	CAGGAGGCCA	AGAAGCTGCT	60
GGGCTCCCTG	CAGCTGCTCT	TTGATAAGAC	GGAGGATGTC	AGCTTCATGA	AGAACACCAA	120
GTCTGTGAAA	ATCCTGATGG	ACAGGACCCA	GACCTGCACG	AGCAGCAAGC	CTTTCCCCCA	180
CTAAGATCGG	CCACCTGAAC	TCCAAGCTTT	CCTGAACGAA	TGGCCAAGAA	GGAGAAANCA	239

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 83 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAAAATCCTG	ATGGACAGGA	CCCAGACCTG	CACGAGCAGC	AGCCTTTCCC	CCACTAAGAT	60
CGGCCACCTG	AACTCCAAGN	TCT				83

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAAAATCCTG	ATGGACAGGA	CCCAGACCTG	CACGAGCAGC	AGCCTTTCCC	CCACTAAGAT	60
CGGCCACCTG	AACTCCAAGC	TCTTCCTGAA	CGAAGGNCCA	AGAAGGAGAA	GCAGCTGCGG	120
AAAATGCTAG	AAGCCCCCTT	CAGCAGCCCG	GTGCCCTTCC	TGCAGAGTGT	CCCCCTGTAC	180
CCTTGCGGCG	TGAGCAGCTC	TGGGGCGGAA	AAGCGCAAGC	ACTCAACGGC	CTTCCCAGAG	240
GCCAGTTTCC	TAGAGACGTC	GTCGGGGCCCT	GTGGGCGC			277

(2) INFORMATION FOR SEQ ID NO:10:

- (A) LENGTH: 114 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCTGCACGNG CAGCAGCCTT TCCCCACTA AGATCGGCCA CCTGAACTCC AAGCTCTTCC 60  
TGAACGAAGG CCCAAGAAGG AGAAGCAGCT GCGGAAAATG CTAGAAGGCC CTTT 114

(2) INFORMATION FOR SEQ ID NO:11:

- (A) LENGTH: 293 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCTGCACGAG	CAGCAGCCTT	TCCCCACTA	AGATCGGCCA	CCTGAACTCC	AAGCTCTTCC	60
TGAACGAATG	GCCAAAGAAGG	AGAAGCAGCT	GCGGAAAATG	CTAGAAGGCC	CCTTCAGCAC	120
GCCGGTGCCC	TTCCTGCAGA	GTGTCCCCCT	GTACCTCTGC	GGCGTAGACA	GCTCTGGGGC	180
GGAAAAGCGC	AAGCACTCAA	CGGCGTTCCT	AGAGGCCAGT	TTCTTAGAGA	CGTCGTCGGG	240
CCCTGTGGGC	GGCCACTTACG	GGGCGGCGGG	CACAGCCAGC	GGTGAGGGCC	AGT	293

(2) INFORMATION FOR SEQ ID NO:12:

- (A) LENGTH: 238 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTCCAAGCTC	TTCCTGAACG	AAGTGTCCTC	GAAGGAGAAG	CAGCTGCGGA	AAATGCTAGA	60
AGGCCCCCTC	AGCACGCCGG	TGCCCTTCCT	GCAGAGTGTC	CCCCTGTACC	CTTGCNGCGT	120
GAGCAGCTCT	GGGGCGGAAA	AGCGCAAGCA	CTCAACGGCC	TTCCAGAGG	CCAGTTTCCT	180
AGAGACGTCG	TCGGGCCCTG	TGGGCGGCCA	GTACGGGGCG	GCGGGCACAG	CCAGCGGT	238

(2) INFORMATION FOR SEQ ID NO:13:

- (A) LENGTH: 284 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGCCGGTGCC	CTTCCTGCAG	AGTGTCCCCC	TGTACCCCTTG	CGGCGTGANA	GCTCTGGGGC	60
GGAAAAGCGC	AAGACTCAAC	GGCCTTCCCA	GAGGCCAGTT	TCCTAGAGAC	GTCGTCGGGC	120
CCTGTGGGCG	GCCAGTACGG	GGCGCGGGG	ACAGGACGCG	GTGAGGGCCA	GTCTGGGCAG	180
CCCCTGGGGC	CCTGCAGCTC	CACGCAGCAA	TTGGTTGGCC	TGCCGGGCGG	GCCTCAACCA	240
GTGCACCTAA	GGCCCGTGTT	CCCCCATCG	CAGTATCCCA	ATGG		284

(2) INFORMATION FOR SEQ ID NO:14:

- (A) LENGTH: 1332 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGAAGANCTG	CTTCGAGGAG	GAGCTCATCT	GCNCTATCTG	CCTGCACGTT	TTCGTGGAGC	60
CAGTGCAGCT	GCCGTGCAAA	CACAACTTCT	GCCGGGGCTG	CATCGGCGAG	GCGTGGGCCA	120
AGGACAGCGG	CCTCGTACGC	TGCCCAGAGT	GCAACCAGGC	CTACAACCAG	AAGCCGGGCC	180
TGGAGAAGAA	CCTGAAGCTC	ACCAACATCG	TGGAGAAGTT	CAATGCCCTG	CACGTGGAGA	240
AGCCGCCGGC	GGCGCTGCAC	TGCGTGTTC	GCCGCCGCGG	CCCCCGCTG	CCCGCGCAGA	300
AGGTCTGCCT	GCGCTGCGAG	GCGCCCTGCT	GCCAGTCCCA	CGTGCAGACG	CACCTGCAGC	360
AGCCCTCCAC	CGCCCGCGGG	CACCTCCTGG	TGGAGGCGGA	CGACGTGCGG	GCCTGGAGCT	420
GCCCGCAGCA	CAACGCCTAC	CGCCTCTACC	ACTGCGAGGC	CGAGCAGGTG	GCCGTGTGCC	480
AGTACTGCTG	CTACTACAGC	GGCGCGCATC	AGGGACACTC	GGTGTGCGAC	GTGGAGATCC	540
GAAGGAATGA	AATCCGGAAG	ATGCTCATGA	AGCAGCAGGA	CCGGCTGGAG	GAGCGAGAGC	600
AGGACATTGA	GGACCAGCTG	TACAAACTCG	AGTCAGACAA	GCGCCTGGTG	GAGGAGAAAG	660
TGAACCAACT	GAAGGAGGAA	GTTTCGGCTGC	AGTACGAGAA	GCTGCACCAG	CTGCTGGACG	720
AGGACCTGCG	GCAGACAGTG	GAGGTCCTAG	ACAAGGCCCA	GGCCAAGTTC	TGCAGCGAGA	780
ACGCAGCGCA	GGCGCTGCAC	CTCGGGGAGC	GCATGCAGGA	GGCCAAGAAG	CTGCTGGGCT	840
CCCTGCAGCT	GCTCTTTGAT	AAGACGGAGG	ATGTCAGCTT	CATGAAGAAC	ACCAAGTCTG	900
TGAAAATCCT	GATGGACAGG	ACCCAGACCT	GCACGAGCAG	CAGCCTTTCC	CCCACTAAGA	960
TCGGCCACCT	GAATCCAAG	CTCTTCCTGA	ACGAAGGGCC	AAGAAGGAGA	AGCAGCTGCG	1020
GAAAATGCTA	GAAGGCCCTT	TCAGCACGCC	GGTGCCCTTC	CTGCAGAGTG	TCCCCCTGTA	1080
CCCTTGCGGC	GTGAGCAGCT	CTGGGGCGGA	AAAGCGCAAG	CACTCAACGG	CCTTCCCAGA	1140
GGCCAGTTTC	CTAGAGACGT	CGTCGGGCCC	TGTGGGCGGC	CAGTACGGGG	CGGCGGGCAC	1200
AGCCAGCGGT	GAGGGCCAGT	CTGGGCAGCC	CCTGGGGCCC	TGCAGCTCCA	CGCAGCAATT	1260
GGTGGCCCTG	CCGGGCGGCG	CCCAACCAGT	GCACTCAAGC	CCCGTGTTC	CCCCATCGCA	1320
GTATCCCAAT	GG					1332

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 68 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGCTCGGAAT	TCCGAGCTTG	GATCCTCTAG	AGCGGCCGCC	GACTAGTGAG	CTCGTCGACC	60
CGGGAATT						68

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 68 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AATTAATTCC	CGGGTCGACG	AGCTCACTAG	TCGGCGGCCG	CTCTAGAGGA	TCCAAGCTCG	60
GAATTCCG						68

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 340 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Lys	Cys	Phe	Glu	Glu	Glu	Leu	Ile	Cys	Ile	Cys	Leu	His	Val	Phe	Val
1				5					10					15	
Glu	Pro	Val	Gln	Leu	Pro	Cys	Lys	His	Asn	Phe	Cys	Arg	Gly	Cys	Ile
			20					25					30		
Gly	Glu	Ala	Trp	Ala	Lys	Asp	Ser	Gly	Leu	Val	Arg	Cys	Pro	Glu	Cys
		35				40					45				
Asn	Gln	Ala	Tyr	Asn	Gln	Lys	Pro	Gly	Leu	Glu	Lys	Asn	Leu	Lys	Leu
	50					55				60					
Thr	Asn	Ile	Val	Glu	Lys	Phe	Asn	Ala	Leu	His	Val	Glu	Lys	Pro	Pro
65					70					75				80	
Ala	Ala	Leu	His	Cys	Val	Phe	Cys	Arg	Arg	Gly	Pro	Pro	Leu	Pro	Ala
				85					90					95	
Gln	Lys	Val	Cys	Leu	Arg	Cys	Glu	Ala	Pro	Cys	Cys	Gln	Ser	His	Val
			100					105					110		
Gln	Thr	His	Leu	Gln	Gln	Pro	Ser	Thr	Ala	Arg	Gly	His	Leu	Leu	Val
	115					120						125			
Glu	Ala	Asp	Asp	Val	Arg	Ala	Trp	Ser	Cys	Pro	Gln	His	Asn	Ala	Tyr
	130					135					140				
Arg	Leu	Tyr	His	Cys	Glu	Ala	Glu	Gln	Val	Ala	Val	Cys	Gln	Tyr	Cys
145					150					155				160	
Cys	Tyr	Tyr	Ser	Gly	Ala	His	Gln	Gly	His	Ser	Val	Cys	Asp	Val	Glu
				165					170					175	
Ile	Arg	Arg	Asn	Glu	Ile	Arg	Lys	Met	Leu	Met	Lys	Gln	Gln	Asp	Arg
			180					185					190		
Leu	Glu	Glu	Arg	Glu	Gln	Asp	Ile	Glu	Asp	Gln	Leu	Tyr	Lys	Leu	Glu
	195					200					205				
Ser	Asp	Lys	Arg	Leu	Val	Glu	Glu	Lys	Val	Asn	Gln	Leu	Lys	Glu	Glu
	210					215				220					
Val	Arg	Leu	Gln	Tyr	Glu	Lys	Leu	His	Gln	Leu	Leu	Asp	Glu	Asp	Leu
225					230					235					
Arg	Gln	Thr	Val	Glu	Val	Leu	Asp	Lys	Ala	Gln	Ala	Lys	Phe	Cys	Ser
				245					250					255	
Glu	Asn	Ala	Ala	Gln	Ala	Leu	His	Leu	Gly	Glu	Arg	Met	Gln	Glu	Ala
		260				265							270		
Lys	Lys	Leu	Gly	Ser	Leu	Gln	Leu	Leu	Phe	Asp	Lys	Thr	Glu	Asp	
		275				280					285				
Val	Ser	Phe	Met	Lys	Asn	Thr	Lys	Ser	Val	Lys	Ile	Leu	Met	Asp	Arg
	290					295				300					
Thr	Gln	Thr	Cys	Thr	Ser	Ser	Ser	Leu	Ser	Pro	Thr	Lys	Ile	Gly	His
305					310					315					320
Leu	Asn	Ser	Lys	Leu	Phe	Leu	Asn	Glu	Gly	Pro	Arg	Arg	Arg	Ser	Ser
				325					330					335	
Cys	Gly	Lys	Cys												
			340												

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 41 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

09350883 094500



Ile Cys Leu His Val Phe Val Glu Pro Val Gln Leu Pro Cys Lys His  
 1 5 10 15  
 Asn Phe Cys Arg Gly Cys Ile Gly Glu Ala Trp Ala Lys Asp Ser Gly  
 20 25 30  
 Leu Val Arg Cys Pro Glu Cys Asn Gln  
 35 40

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

His Cys Val Phe Cys Arg Arg Gly Pro Pro Leu Pro Ala Gln Lys Val  
 1 5 10 15  
 Cys Leu Arg Cys Glu Ala Pro Cys Cys Gln Ser His Val Gln Thr His  
 20 25 30  
 Leu Gln Gln Pro Ser Thr Ala Arg  
 35 40

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Trp Ser Cys Pro Gln His Asn Ala Tyr Arg Leu Tyr His Cys Glu Ala  
 1 5 10 15  
 Glu Gln Val Ala Val Cys Gln Tyr Cys Cys Tyr Tyr Ser Gly Ala His  
 20 25 30  
 Gln Gly His Ser Val Cys Asp  
 35

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asp Arg Thr Gln Thr Cys Thr Ser Ser Ser Leu Ser Pro Thr Lys Ile  
 1 5 10 15  
 Gly His Leu Asn Ser Lys Leu Phe Leu Asn Glu Gly Pro Arg Arg Arg  
 20 25 30  
 Ser Ser Cys Gly Lys Cys  
 35

(2) INFORMATION FOR SEQ ID NO:22:

05250883 021599

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Asp Tyr Lys Asp Asp Asp Asp Lys  
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Met His Thr Glu His  
1 5 10 15  
His His His His His  
20

We Claim:

1. A method of detecting the presence of a target BS203 polynucleotide in a test sample, comprising:
- 5 (a) contacting said test sample with at least one BS203-specific polynucleotide or complement thereof; and
- (b) detecting the presence of said target BS203 polynucleotide in the test sample, wherein said BS203-specific polynucleotide has at least 50% identity to a
- 10 polynucleotide selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, SEQUENCE ID NO 6, SEQUENCE ID NO 7, SEQUENCE ID NO 8, SEQUENCE ID NO 9, SEQUENCE ID NO 10, SEQUENCE ID NO 11, SEQUENCE ID NO 12, SEQUENCE ID NO 13, SEQUENCE ID NO 14, and
- 15 fragments or complements thereof.
2. The method of claim 1, wherein said target BS203 polynucleotide is attached to a solid phase prior to performing step (a).
3. A method of detecting a target BS203 polynucleotide in a test sample suspected of containing said target, comprising:
- 20 (a) contacting said test sample with at least one BS203 oligonucleotide as a sense primer and with at least one BS203 oligonucleotide as an anti-sense primer and amplifying to obtain a first stage reaction product;
- 25 (b) contacting said first stage reaction product with at least one other BS203 oligonucleotide to obtain a second stage reaction product, with the proviso that the other BS203 oligonucleotide is located 3' to the BS203 oligonucleotides utilized in step (a) and is complementary to said first stage reaction product; and
- (c) detecting said second stage reaction product as an indication of the
- 30 presence of the target BS203 polynucleotide, wherein the BS203 oligonucleotides utilized in step (a) and step (b) have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, SEQUENCE ID NO 6, SEQUENCE ID NO 7, SEQUENCE ID NO 8, SEQUENCE ID NO 9, SEQUENCE ID NO 10, SEQUENCE ID NO 11, SEQUENCE ID NO 12, SEQUENCE ID NO
- 35 13, SEQUENCE ID NO 14, and fragments or complements thereof.

4. The method of claim 3, wherein said test sample is reacted with a solid phase prior to performing one of steps (a), (b) or (c).

5 5. The method of claim 3, wherein said detection step comprises utilizing a detectable label capable of generating a measurable signal.

6. The method of claim 5, wherein said detectable label is reacted to a solid phase.

10

7. A method for detecting BS203 antigen in a test sample suspected of containing said BS203 antigen, comprising:

(a) contacting the test sample with an antibody or fragment thereof which specifically binds to at least one epitope of a BS203 antigen selected from the group consisting of SEQUENCE ID NO 17, SEQUENCE ID NO 18, SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21, and fragments thereof, wherein said contacting is carried out for a time and under conditions sufficient for the formation of antibody/antigen complexes; and

15 (b) detecting the presence of said complexes as an indication of the presence of said BS203 antigen.

20

8. The method of claim 7, wherein said antibody is attached to a solid phase.

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REAGENTS AND METHODS USEFUL FOR  
DETECTING DISEASES OF THE BREAST

5

Abstract of the Disclosure

10 A set of contiguous and partially overlapping RNA sequences and  
polypeptides encoded thereby, designated as BS203 and transcribed from breast  
tissue is described. These sequences are useful for the detecting, diagnosing,  
staging, monitoring, prognosticating, preventing or treating, or determining the  
predisposition of an individual to diseases and conditions of the breast such as breast  
cancer. Also provided are antibodies which specifically bind to BS203-encoded  
15 polypeptide or protein, and agonists or inhibitors which prevent action of the tissue-  
specific BS203 polypeptide, which molecules are useful for the therapeutic treatment  
of breast diseases, tumors or metastases.

09250083 034509  
003720 0005260

# Figure 1-A

>2269559	GGAAGANCTG	CTTCGAGGAG	GAGCTCATCT	GCNCTATCTG	CCTGCACGTT
>1664718					GTT
Consensus	GGAAGANCTG	CTTCGAGGAG	GAGCTCATCT	GCNCTATCTG	CCTGCACGTT
>2269559	TTCGTGGAGC	CAGTGCAGCT	GCCGTGCAAA	CACANCTTCT	GCCGGGGCTG
>1664718	TTCGTGGAGC	CAGTGCAGCT	GCCGTGCAAA	CACAACCTTCT	GCCGGGGCTG
Consensus	TTCGTGGAGC	CAGTGCAGCT	GCCGTGCAAA	CACAACCTTCT	GCCGGGGCTG
>2269559	CATCGGCGAG	GCGTGGGCCA	AGGACAGCGG	CCTCGTACGC	TGCCCAGAGT
>1664718	CATCGGCGAG	GCGTGGGCCA	AGGACAGCGG	CCTCGTACGC	TGCCCAGAGT
>2360586	ATCGGCGAG	GCGTGGGCCA	AGGACAGCNG	CCTCGTACGC	TGCCCAGAGT
Consensus	CATCGGCGAG	GCGTGGGCCA	AGGACAGCGG	CCTCGTACGC	TGCCCAGAGT
>2269559	GCAACCAGGC	CTACAACCAG	ANGCCGGGCC	TGGAGAAGAA	CCTGAAGCTC
>1664718	GCAACCAGGC	CNACAACCAG	AAGCCGGGCC	TGGNGAAGAA	CCTGAAGCTC
>2360586	GCAACCAGGC	CTACAACCAG	AAGCCGGGCC	TGGAGAAGAA	CCTGAAGCTC
Consensus	GCAACCAGGC	CTACAACCAG	AAGCCGGGCC	TGGAGAAGAA	CCTGAAGCTC
>2269559	ACCAACATCG	TGGAGAAGTT	CAATGCCCTG	CNCGTGGAGA	NGCCGCCGGC
>1664718	ACCAACATCG	TGGAGAAGTT	CAATGCCCTG	CACGTGGAGA	ANCCGCCGGC
>2360586	ACCAACATCG	TGGAGAAGTT	CAATGCCCTG	CACGTGGAGA	NGCCGCCGGC
Consensus	ACCAACATCG	TGGAGAAGTT	CAATGCCCTG	CACGTGGAGA	AGCCGCCGGC
>2269559	GGCGCTGCAC	TG			
>1664718	GGCGCTGCAC	TGCGTGTCT	GCCGCCGCGG	CCCCCGCTG	CCCGCGCAGA
>2360586	NGCGCTGCAC	TGCGTGTCT	GCCGCCGCGG	CCCCCGCTG	CCCGCGCAGA
Consensus	GGCGCTGCAC	TGCGTGTCT	GCCGCCGCGG	CCCCCGCTG	CCCGCGCAGA
>1664718	AGGTCTGCCT				
>2360586	AGGTCTGCCT	GCGCTGCGAG	GCGCCCTGCT	GCCAGTCCCA	CGTGCAGACG
>1436565		T GCGCTGCGAG	GCGCCCTGCT	GCCAGTCCCA	CGTGCAGACG
Consensus	AGGTCTGCCT	GCGCTGCGAG	GCGCCCTGCT	GCCAGTCCCA	CGTGCAGACG
>2360586	CACCT				
>1436565	CACCTGCAGC	AGCCCTCCAC	CGCCCGCGGG	CACCTCCTGG	TGGAGGCGGA
Consensus	CACCTGCAGC	AGCCCTCCAC	CGCCCGCGGG	CACCTCCTGG	TGGAGGCGGA
>1436565	CGACGTGCGG	GCCTGGAGCT	GCCCGCAGCA	CAACGCCTAC	CGCCTCTACC
Consensus	CGACGTGCGG	GCCTGGAGCT	GCCCGCAGCA	CAACGCCTAC	CGCCTCTACC
>1436565	ACTGCGAGGC	CGAGCAGGTG	GCCGTGTGCC	AGTACTGCTG	CTACTACAGC
Consensus	ACTGCGAGGC	CGAGCAGGTG	GCCGTGTGCC	AGTACTGCTG	CTACTACAGC
>1436565	GGCGCGCATC	AGGGACACTC	GGTGTGCGAC	GTGGAGATCC	GAAGGAATGA
>2479125			GGTGTGCGAC	GTGGAGATCC	GAAGGAATGA
Consensus	GGCGCGCATC	AGGGACACTC	GGTGTGCGAC	GTGGAGATCC	GAAGGAATGA
>1436565	AATCCGGAAG	ATGCTCATGA	AGCAGCAGGA	CC	
>2479125	AATCCGGAAG	ATGCTCATGA	AGCAGCAGGA	CCGGCTGGAG	GAGCGAGAGC
Consensus	AATCCGGAAG	ATGCTCATGA	AGCAGCAGGA	CCGGCTGGAG	GAGCGAGAGC
>2479125	AGGACATTGA	GGACCAGCTG	TACAAACTCG	AGTCAGACAA	GCGCCTGGTG
>2247228					GTG
Consensus	AGGACATTGA	GGACCAGCTG	TACAAACTCG	AGTCAGACAA	GCGCCTGGTG

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## Figure 1-B

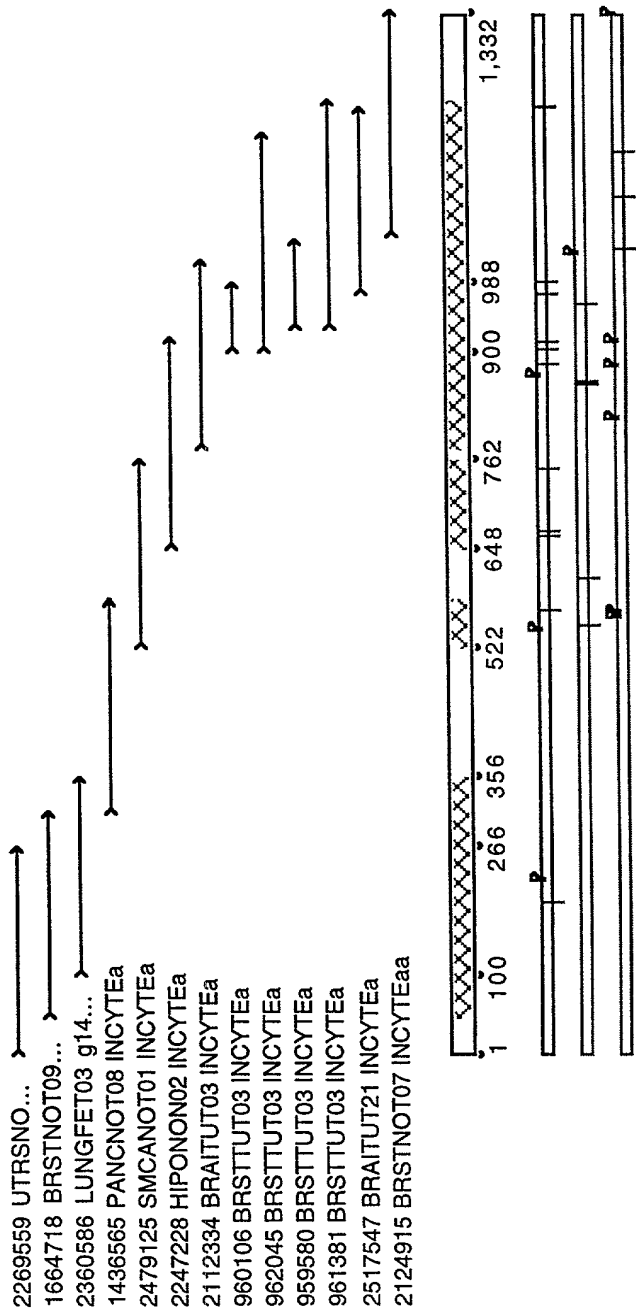
>2479125	GAGGAGAAAG	TGAACCAACT	GAAGGAGGAA	GTTCCGGCTGC	AGTACGAGAA
>2247228	GAGGAGAAAG	TGAACCAACT	GAAGGAGGAA	GTTCCGGCTGC	AGTACGAGAA
Consensus	GAGGAGAAAG	TGAACCAACT	GAAGGAGGAA	GTTCCGGCTGC	AGTACGAGAA
>2479125	GCTGCACCAG	CTGCTGGACG	AGGACCTGCG	GCAGACAGTG	GAGGTCCTAG
>2247228	GCTGCACCAG	CTGCTGGACG	AGGACCTGCG	GCAGACAGTG	GAGGTCCTAG
Consensus	GCTGCACCAG	CTGCTGGACG	AGGACCTGCG	GCAGACAGTG	GAGGTCCTAG
>2479125	ACAAGGCCCA				
>2247228	ACAAGGCCCA	GGCCAAGTTC	TGCAGCGAGA	ACGCAGCGCA	GGCGCTGCAC
>2112334			CGAGA	ACGCAGCGCA	GGCGCTGCAC
Consensus	ACAAGGCCCA	GGCCAAGTTC	TGCAGCGAGA	ACGCAGCGCA	GGCGCTGCAC
>2247228	CTCGGGGAGC	GCATGCAGGA	GGCCAAGAAG	CTGCTGGGCT	CCCTGCAGCT
>2112334	CTCGGGGAGC	GCATGCAGGA	GGCCAAGAAG	CTGCTGGGCT	CCCTGCAGCT
Consensus	CTCGGGGAGC	GCATGCAGGA	GGCCAAGAAG	CTGCTGGGCT	CCCTGCAGCT
>2247228	GCTCTTTGAT	AAGACGGAGG	ATGTCAGCTT	CATGAAGAAC	ACCAAGTCTG
>2112334	GCTCTTTGAT	AAGACGGAGG	ATGTCAGCTT	CATGAAGAAC	ACCAAGTCTG
Consensus	GCTCTTTGAT	AAGACGGAGG	ATGTCAGCTT	CATGAAGAAC	ACCAAGTCTG
>2247228	TGAAAATCCT	GATGG			
>2112334	TGAAAATCCT	GATGGACAGG	ACCCAGACCT	GCACGAGCAG	CAAGCCTTTC
>960106	GAAAATCCT	GATGGACAGG	ACCCAGACCT	GCACGAGCAG	CA:GCCTTTC
>962045	GAAAATCCT	GATGGACAGG	ACCCAGACCT	GCACGAGCAG	CA:GCCTTTC
>959580			CCT	GCACGNGCAG	CA:GCCTTTC
>961381			CCT	GCACGAGCAG	CA:GCCTTTC
Consensus	TGAAAATCCT	GATGGACAGG	ACCCAGACCT	GCACGAGCAG	CA:GCCTTTC
>2112334	CCCCACTAAG	ATCGGCCACC	TGAACTCCAA	GCT:TTCCTG	AACGAATGG:
>960106	CCCCACTAAG	ATCGGCCACC	TGAACTCCAA	GNTCT	
>962045	CCCCACTAAG	ATCGGCCACC	TGAACTCCAA	GCTCTTCCTG	AACGAAGGN:
>959580	CCCCACTAAG	ATCGGCCACC	TGAACTCCAA	GCTCTTCCTG	AACGAAGGC:
>961381	CCCCACTAAG	ATCGGCCACC	TGAACTCCAA	GCTCTTCCTG	AACGAATGG:
>2517547			CTCCAA	GCTCTTCCTG	AACGAAGTGT
Consensus	CCCCACTAAG	ATCGGCCACC	TGAACTCCAA	GCTCTTCCTG	AACGAAGGG:
>2112334	CCAAGAAGGA	GAANCA			
>962045	CCAAGAAGGA	GAAGCAGCTG	CGGAAAATGC	TAGAAGGCCC	CTTCAGCACG
>959580	CCAAGAAGGA	GAAGCAGCTG	CGGAAAATGC	TAGAAGGCCC	CTT
>961381	CCAAGAAGGA	GAAGCAGCTG	CGGAAAATGC	TAGAAGGCCC	CTTCAGCACG
>2517547	CCAAGAAGGA	GAAGCAGCTG	CGGAAAATGC	TAGAAGGCCC	CTTCAGCACG
>2124915					CG
Consensus	CCAAGAAGGA	GAAGCAGCTG	CGGAAAATGC	TAGAAGGCCC	CTTCAGCACG
>962045	CCGGTGCCCT	TCCTGCAGAG	TGTCCCCCTG	TACCCTTGCG	GCGTGAGCAG
>961381	CCGGTGCCCT	TCCTGCAGAG	TGTCCCCCTG	TACCCTTGCG	GCGTGAGCAG
>2517547	CCGGTGCCCT	TCCTGCAGAG	TGTCCCCCTG	TACCCTTGCG	GCGTGAGCAG
>2124915	CCGGTGCCCT	TCCTGCAGAG	TGTCCCCCTG	TACCCTTGCG	GCGTGAN:AG
Consensus	CCGGTGCCCT	TCCTGCAGAG	TGTCCCCCTG	TACCCTTGCG	GCGTGAGCAG

## Figure 1-C

>962045	CTCTGGGGCG	GAAAAGCGCA	AGCACTCAAC	GGCCTTCCCA	GAGGCCAGTT
>961381	CTCTGGGGCG	GAAAAGCGCA	AGCACTCAAC	GGCCTTCCCA	GAGGCCAGTT
>2517547	CTCTGGGGCG	GAAAAGCGCA	AGCACTCAAC	GGCCTTCCCA	GAGGCCAGTT
>2124915	CTCTGGGGCG	GAAAAGCGCA	AG:ACTCAAC	GGCCTTCCCA	GAGGCCAGTT
Consensus	CTCTGGGGCG	GAAAAGCGCA	AGCACTCAAC	GGCCTTCCCA	GAGGCCAGTT
>962045	TCCTAGAGAC	GTCGTCGGGC	CCTGTGGGCG		
>961381	TCCTAGAGAC	GTCGTCGGGC	CCTGTGGGCG	GCCAGTACGG	GGCGGCGGGC
>2517547	TCCTAGAGAC	GTCGTCGGGC	CCTGTGGGCG	GCCAGTACGG	GGCGGCGGGC
>2124915	TCCTAGAGAC	GTCGTCGGGC	CCTGTGGGCG	GCCAGTACGG	GGCGGCGGGC
Consensus	TCCTAGAGAC	GTCGTCGGGC	CCTGTGGGCG	GCCAGTACGG	GGCGGCGGGC
>961381	ACAGCCAGCG	GTGAGGGCCA	GT		
>2517547	ACAGCCAGCG	GT			
>2124915	ACAGCCAGCG	GTGAGGGCCA	GTCTGGGCAG	CCCCTGGGGC	CCTGCAGCTC
Consensus	ACAGCCAGCG	GTGAGGGCCA	GTCTGGGCAG	CCCCTGGGGC	CCTGCAGCTC
>2124915	CACGCAGCAA	TTGGTGGCCC	TGCCGGGCGG	CGCCCAACCA	GTGCACTCAA
Consensus	CACGCAGCAA	TTGGTGGCCC	TGCCGGGCGG	CGCCCAACCA	GTGCACTCAA
>2124915	GCCCCGTGTT	CCCCCCATCG	CAGTATCCCA	ATGG	
Consensus	GCCCCGTGTT	CCCCCCATCG	CAGTATCCCA	ATGG	



Figure 2



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Maurice Cohen, Paula N.  
Friedman, Michael R. Klass, Lisa Roberts-  
Rapp and John C. Russell

Serial No.: 1.53(b) of 08/889,316

Filed: on even date herewith

Title: REAGENTS AND METHODS  
USEFUL FOR DETECTING DISEASES OF  
THE BREAST

Case No.: 6131.US.02

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Washington, D.C. 20231

on

February 16, 1999

Date

Patricia K. Himenes

Patricia K. Himenes

**DECLARATION AND POWER OF ATTORNEY FOR A UNITED STATES PATENT APPLICATION**

Assistant Commissioner for Patents

**Box Patent Application**

Washington, D.C. 20231

As a below-named inventor, I hereby declare that my residence, post office address and citizenship are as  
stated below next to my name. I believe that I am an original and first and joint inventor of the subject matter of  
the invention entitled REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE BREAST,  
which is claimed and for which a patent is sought in the patent application attached hereto.

I hereby state that I have reviewed and understand the contents of the above-mentioned specification, including  
the claims.

I acknowledge a duty to disclose to the Patent and Trademark Office all information known to me to be material  
to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

Claim to benefit of foreign application(s):

I hereby claim foreign priority benefits under 35 U.S.C. §119 for the following foreign application(s)  
for patent or inventor's certificate:

NONE

The following foreign applications for patent or inventor's certificate have a filing date earlier than the  
filing date of the application(s) identified above:

NONE

Claim to benefit of U.S. Application(s):

I hereby claim the benefit under 35 U.S.C. §120 of the following earlier-filed United States patent application(s):

08/889,316 filed July 8, 1997.

Insofar as the subject matter of each of the claims of this/these application(s) is not disclosed in the prior U.S. Applications in the manner required by 35 U.S.C. § 112, first paragraph, I acknowledge a duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. Which came into existence between the filing date(s) of the prior applications and the national or PCT filing date of this application.

I hereby appoint the following Attorneys and/or agents to prosecute this application and any continuation or divisional applications based hereon, and to transact all business in the Patent and Trademark Office connected therewith:

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Nicholas A. Poulos, Reg. No. 30,209  
Gregory W. Steele, Reg. No. 33,796  
Michael J. Ward, Reg. No. 37,960  
David L. Weinstein, Reg. No. 28,128  
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Residence: Kenosha, WI 53142

Citizenship: US

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made herein were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

\_\_\_\_\_  
Maurice COHEN

\_\_\_\_\_  
Paula N. FRIEDMAN

\_\_\_\_\_  
Michael R. KLASS

\_\_\_\_\_  
Lisa ROBERTS-RAPP

\_\_\_\_\_  
John C. RUSSELL

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: J. C. Russell *et al.*

Serial No.: 08/889,316

Filed: July 8, 1997

Title: REAGENTS AND METHODS  
USEFUL FOR DETECTING  
DISEASES OF THE BREAST

Case No.: 6131.US.01

Examiner: not known

Group Art Unit: 1807

Certificate of Mailing Under 37 C.F.R. §1.8(a)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as First Class Mail in an envelope addressed to:

Attention: Box Missing Parts  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Date of Deposit: November 19, 1997

*Margaret Pendergast* 11-19-97  
Margaret Pendergast Date

DECLARATION AND POWER OF ATTORNEY FOR A UNITED STATES PATENT APPLICATION

Attention: Box Missing Parts  
Assistant Commissioner for Patents  
Washington, D.C. 20231

My residence, post office address and citizenship are as stated below next to my name. I believe I am an original and first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE BREAST the specification which has been given U.S. Serial No. 08/889,316, and accorded the filing date of July 8, 1997.

I hereby state that I have reviewed and understand the contents of the above-mentioned specification, including the claims.

I acknowledge a duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

Claim to benefit of foreign application(s):

I hereby claim foreign priority benefits under 35 U.S.C. §119 for the following foreign application(s) for patent or inventor's certificate:

NONE

The following foreign applications for patent or inventor's certificate have a filing date earlier than the filing date of the application(s) identified above:

NONE

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659120" 6590566

**Claim to benefit of earlier U.S. application(s):**

I hereby claim the benefit under 35 U.S.C. §120 of the following earlier-filed United States patent application(s):

NONE

In so far as the subject matter of each of the claims of this/these application(s) is not disclosed in the prior U.S. applications in the manner required by 35 U.S.C. §112, first paragraph, I acknowledge a duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. which came into existence between the filing date(s) of the prior applications and the national or PCT filing date of this application.

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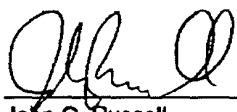
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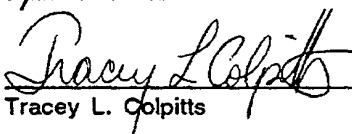
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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made herein were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

  
John C. Russell      11/7/97  
Date

  
Tracey L. Colpitts      11/7/97  
Date